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CONTENTS OF VOL. XV, No. 2

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EXPERIMENTS WITH A MOSAIC DISEASE OF TOMATO

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(With Plate IX.)

Description of Virus. In the experiments described in the following pages a virus was used which produces in the tomato a mosaic disease somewhat different in appearance from the usual tomato or tobacco mosaic. It was given to the writer in 1925 by Dr W. F. Bewley, who had found it on tomato two or three years previously, and had given it the name of Aucuba mosaic of tomato from its resemblance (not, it must be admitted, very close) to Aucuba mosaic of the potato. In its general characters as well as in the nature of the disease it produces, it corresponds very closely with the virus of tomato or tobacco mosaic, but it differs from the latter in the much greater intensity and brilliance of the leaf-symptoms. Since its first isolation it has been maintained regularly on tomato without change in these symptoms, and the difference, while possibly only one of degree, is so striking that it is difficult to avoid the conclusion that there is also a difference in the two viruses.

It seems probable that this mosaic is the same as the "Yellow Tobacco Mosaic" (Tobacco Virus 6) first described by Johnson in 1927 (8) and again referred to by Hoggan (7). The characters of the virus, so far as they are known, are the same in both, and the signs produced by yellow mosaic on tomato and tobacco, as shown in the figures in Johnson's paper, resemble very closely those produced by the virus here described. Without further work, however, and until a more satisfactory system of virus classification has been established, it is impossible to be certain that the two are identical.

While the signs on the plant vary somewhat with the variety of tomato and still more with external conditions of temperature and light, and the rate of growth, the following description of it as it occurs on Kondine Red tomatoes is fairly typical of the disease in general. When the plants are very young at the time of inoculation, *i.e.* with only about three leaves large enough to be readily inoculated, and the

conditions are favourable for rapid growth, the first signs appear in the young developing leaves of the crown about the 5th day. These show a downward curling of the whole leaf, with slight turning down of the margins, and the surface of the leaf is rough, wrinkled or corrugated. The colour is still green with no sign of chlorosis or mottling, but the evidence of abnormal growth is quite definite. By the 7th or 8th day points of chlorosis appear on these curled leaves, sometimes at the base of the leaflet, more usually at the tips and margins, and these rapidly increase in number, are distributed over the whole surface of the leaf, and tend to coalesce. By the 12th or 13th day, when usually six more leaves have unfolded, the signs of disease differ in the different leaves. The original three may still show no chlorosis, or only a slight yellowing of the veins, and the youngest leaf may also be quite green. But the fourth and fifth and sometimes the sixth, *i.e.* the leaves developing after inoculation, are extensively affected. In extreme cases almost the whole surface is pale yellow to white with here and there small islets of intense dark green, which stand up as small blisters. In less extreme cases the green areas are larger, but as a rule the area of white or pale yellow is greater than the green area. The surface is uneven and there may be turning down of tip and margins. These three leaves show the most extreme form of the disease which will be seen in the whole plant. The younger leaves at this time show only scattered patches of white or yellow, frequently angular or triangular at vein intersections, and the youngest leaf may be entirely green. As growth proceeds each leaf in turn may come out with only slight colour changes, but later on each develops more or less extensive signs, though rarely in the later leaves is the chlorosis so extensive as in the fourth to the sixth leaves. When the plant reaches a height of 18 to 24 in., by which time the first flowers are forming, a typical leaf will show on each leaflet areas of four different shades. Most of the surface is green, partly of normal tint and partly of a deeper and richer shade. Scattered over the leaf are patches of white and patches of yellow, usually sharply delineated but sometimes shading into neighbouring areas, irregular in shape and size, often angular, and occurring in all parts of the leaf (Plate IX, fig. 1).

The plant is not killed, but goes on to the production of fruit, which may or may not be mottled. But its growth is checked: compared with normal plants of the same age, it is stunted and of spindling habit. There is no necrosis. Sometimes the extensive chlorotic areas, especially in the fourth to sixth leaves, dry out, and turn a dull brown, but necrosis is not a character of the disease, and in many cases even this secondary

bronzing does not occur. There is little tendency to extreme malformation, though quite definite fern-leaf has been noted occasionally on plants growing rather slowly, *e.g.* in the autumn.

In atypical cases, or when incubation is unusually prolonged or the disease is less acute, the leaves may show at first a yellowing of the veins, which thereby become more conspicuous, and appear as a yellow network on a green background (Plate IX, fig. 2). As a rule these leaves later develop intervenal signs. If the plant is already well grown, *i.e.* with flowers already out, at the time of inoculation, the signs appear on the younger leaves, and not on the older parts, incubation is prolonged to 14 days or more, and there is not the extensive whitening seen on the plant which has been infected young. The signs are, however, perfectly clear and definite, and of the same character as those in the later-developing leaves of the plant infected young.

From this description it will be seen that the course of the disease resembles very closely that of the usual mosaic on tomato or tobacco. The character of the symptoms also on the whole resembles the more common disease, and, as is shown later, this virus is like the ordinary mosaic in its filterability, resistance to heat, to alcohol, to dilution and to ageing, and in its ready transmissibility by inoculation of juice or tissue as well as by insects. But the actual picture presented by the Aucuba or yellow type is much more striking, more spectacular, than any which the writer has seen with the usual mosaic. The white areas are more intensely white, the green areas more vividly contrasted, and there is usually a sharper delimitation between the two. When the two types are seen side by side, the difference is very conspicuous. Essentially, however, the two diseases are the same in kind throughout, and the Aucuba type has been used in these experiments as a typical mosaic disease, because it is very characteristic, easily recognisable, and not liable to be confused with the mottlings due to physiological or environmental conditions or with possible contamination by other types of mosaic.

Methods of Inoculation. Inoculation was made at first by simple pricking with a needle through juice dropped on the leaf, a total of 80–100 punctures being made on at least three leaves per plant. In later work this method has been modified by supporting the leaf on the wooden slip used to mark the pot, dropping on it the inoculum, and then scratching through the drop with the point of a needle in a number of places, usually about 30 per leaf and always inoculating at least three leaves. This method avoids all contact of the hands with the leaf or

inoculum, and is effective. The weakness of all such methods is that one has very little idea of the amount actually inoculated and not much assurance that any two plants have received the same dose, whatever its size. The variation, however, can hardly be greater than 100 per cent., and an accuracy of this order is as great as is necessary in most work.

In a number of cases infection was carried out in a different manner. A petiole was cut across, and the cut end dipped into a small phial containing the juice to be inoculated. The plant absorbs the contents of the phial through the cut surface, and in this way a definite dose can be introduced. But the method is too laborious for use with large numbers of plants. It is also rather uncertain, since one plant may take up in one hour as much as 1 c.c., while another takes up only 0·2 c.c., and sometimes only quite small quantities may be taken up in 18 hours. Further, it would seem to be less efficient. In one series of four plants absorption of 0·5 c.c. of filtered juice by each, and in another series of six plants absorption of 0·7 c.c. by each failed to produce the disease in any, while in a third series only 50 per cent. of the plants took the disease as against 83 and 100 per cent. in two other series done at the same time with different methods.

Method of Filtration. The method adopted to obtain filtered juice is as follows. Leaves and succulent parts of the stem of infected plants are weighed, minced with scissors, and ground in a mortar without sand, distilled water added gradually with renewed grinding in the quantity of 3 c.c. of water to 1 gm. of tissue, and the mass squeezed by hand through muslin. The resulting turbid green liquid is then passed, under pressure, through a cylinder tightly packed with alternate layers of sand and paper-pulp[1], which gives a perfectly clear brown fluid. (At one time instead of the cylinder filter-paper was used, the liquid being passed through the same paper more than once, but this was a slow and uncertain process.) The clear fluid is then passed through first a "L 1," and then at once through a "L 3" Pasteur-Chamberland filter; and the filtered fluid immediately distributed in small volumes (5 or 10 c.c.) into test-tubes or flasks, and remains bacteriologically sterile. The whole process from cutting the plant to final distribution of 150–250 c.c. of fluid requires about 4 hours: all apparatus having been previously sterilised. Unless otherwise stated, "filtered juice" in this paper means juice prepared in this way. From 100 gm. of tissue to which 300 c.c. of water are added, 330–340 c.c. of crude liquid are obtained after

[1] This should be made from macerated ashless filter-paper; the usual commercial compressed pulp alters the reaction of the juice.

squeezing through muslin. Some liquid, of course, remains in the mass of tissue, but one may reckon that the juice is diluted 1 in 8 to 1 in 10 before passing through the candles.

Effect of Dilution. In Table I is shown an experiment to determine to what extent filtered juice can be diluted and still remain infective. The dilution was made with distilled water, a fresh pipette being used for each step. It will be seen that 1 in 1000 is still fully infective; 1 in 10,000 infective but less markedly; with 1 in 100,000 or weaker strengths infection did not occur. This is the usual result with juice obtained from young plants grown under standard conditions and with the signs of disease well-marked, about 4 weeks after infection; but some variation occurs under other conditions, *e.g.* when the plants are less succulent or older. Also, the 1 in 100 dilution may not always produce 100 per cent.

Table I.

Filtered juice diluted in distilled water.

Dilution	No. of plants	No. positive	% positive
$1 : 10^2$	8	8	100
$1 : 10^3$	8	8	100
$1 : 10^4$	8	3	37·5
$1 : 10^5$	8	0	0
$1 : 10^6$	8	0	0

infection, not even when a dilution of 1 in 10,000 is still partially infective; but with 1 in 100 dilution infection of over 80 per cent. was always obtained during the season of the year favourable to growth. These results correspond well with those obtained with the usual tobacco mosaic virus. With it, and using unfiltered juice, infection may still be got with dilutions of 1 : 100,000 or even $1 : 10^6$ (Allard(1)), but filtration always reduces notably the extent to which dilution is practicable.

Resistance to Heat and Ageing. The filtered juice withstands heating for 10 minutes at 80° C. but is no longer infective after exposure for the same time at 90° C. (Table II). These limits are no doubt subject to a certain amount of variation, according to the composition of the particular juice or other factors, and also according to the concentration of virus in the sample under test. No definite thermal death-point is possible, since there is involved a time-factor which varies with the concentration of the material undergoing destruction. But with standardised plants and uniform methods inactivation occurs regularly between 80° C. and 90° C. in 10 minutes. No attempt has been made to obtain more precise determination.

Table II.

2·5 c.c. samples of filtered juice in thin-walled tubes, with thermometer attached, introduced into water-baths at the temperatures named, and withdrawn to cold water after 10 minutes. Great care was taken to ensure that the whole sample was deeply immersed.

Temp. (° C.)	No. of plants	No. positive	% positive
50	7	7	100
60	7	7	100
70	8	8	100
80	8	8	100
90	8	0	0

Similar results have been obtained with tobacco mosaic in tobacco juice by various observers. For example, Mulvania (11) found that with 10 minutes exposure 80° C. reduced the infectivity not at all, 83° C. to 80 per cent., 85° C. to 50 per cent., 89° C. to 10 per cent., while 90° C. abolished it. (See also Allard (2) and McKinney (9).) With tomato mosaic Walker (16) found the infectivity destroyed in 10 minutes between 85° C. and 90° C., though on one occasion juice was still infective after 95° C. for the same time.

Filtered juice is still infective after being kept in subdued light at room temperature for one year or more.

Resistance to Alcohol. The virus is not destroyed by 90 per cent. alcohol after one hour's contact at room temperature—see Table III. In these determinations absolute alcohol was added to filtered juice in sufficient quantity to give the desired concentration, the volume of juice being the same in all series. After thorough mixing, the vessel was corked and left for one hour at room temperature, and then the mixture was centrifuged for 30 minutes. The supernatant liquid was pipetted off, and tested separately; to the deposit was added distilled water equal in volume to that of the original juice, and after thorough mixing the liquid was inoculated. As Table III shows, the deposit remains fully active even from 90 per cent. alcohol. The supernatant liquid, however, remained slightly active after 70 per cent. alcohol treatment, and still more after 60 per cent. This is not in agreement with Allard's work (2), who with tobacco mosaic found the supernatant inactive after precipitation with 45 per cent. or stronger alcohol. The discrepancy is due probably, not to a difference in method, but to imperfect sedimentation in my experiments, the available centrifuge running at low speed. This is suggested by the variability in the result, as shown in Table III, and also by the fact that when the supernatant was passed through a "L 3" candle, it was quite inactive, even from 50 per cent. alcohol, probably

because the filter removed small flocculi which had not come down in the centrifuge (cf. Olitsky and Boez(13)). Even after 4 days' contact with 60 per cent. alcohol, the virus remained fully active, giving 100 per cent. infection, and repeated washing of the precipitate with 60 per cent. alcohol did not reduce its infectivity.

Walker(16), using tomato mosaic juice, found the precipitate infective after one hour's treatment with alcohol in all concentrations from 33 to 95 per cent. Allard(2), on the other hand, found the precipitate not infective after 1–2 days' contact with 75 or 80 per cent. alcohol, using tobacco mosaic from tobacco; and cucumber mosaic in cucumber juice cannot withstand even 45 per cent. (Doolittle(3)).

Table III.

Effect of Alcohol.

A. After 1 hour's contact and then centrifugalisation.

Concentration alcohol %	Precipitate		Supernatant unfiltered		Supernatant filtered	
	No. of plants	No. positive	No. of plants	No. positive	No. of plants	No. positive
50	.	.	.	.	8	0
60	8	8	8	6	8	0
60	8	8	8	2	8	0
70	8	8	8	1	.	.
80	8	8	8	0	.	.
90	8	8	.	.	.	.

B. After 4 days' contact with 60 % alcohol : precipitate: 8 plants, 8 positive.

This high resistance to alcohol of certain plant viruses is a remarkable phenomenon, and the only parallel to it to be found in the literature of other virus diseases is in the case of the virus of foot and mouth disease of cattle. But even here the resistance appears to be of a lower order. The English Commission on this disease(4) found that the virus occasionally withstood 60 per cent. alcohol for 18 hours, but never longer in their experience; and the resistance varied with different samples of virus, some being inactivated after 5–6 hours' contact. The supernatant liquid also occasionally remained infective. Olitsky and Boez(13) found that the foot and mouth virus with which they worked resisted 60 per cent. alcohol for 26 hours or more, and that the supernatant liquid was always inactive if care were taken to remove from it, by filtration or otherwise, all undeposited flocculi of precipitate. They believed, however, that this resistance is not a genuine property of the virus. In their view, the virus is really sensitive, but the precipitate (of

protein or other material present in the liquid) produced by the alcohol protects the virus from its action; and, when the formation of this precipitate is prevented, *e.g.* by modification of the reaction of the liquid by addition of sodium hydrate, the virus is killed in a very short time (a minute or two) and shows no more resistance than *Bacillus coli* or *Staphylococcus*. It seemed desirable to repeat this work with a plant virus.

Preliminary experiments showed that to prevent the formation in tomato juice of visible precipitate on the addition of alcohol to 60 per cent., considerable quantities of NaOH are necessary. Even with 1·0 c.c. of *N*/1 NaOH solution and alcohol to 60 per cent. precipitation was usually visible within half-an-hour of adding 5 c.c. of juice, although small in amount and delayed in formation. With 1·3 c.c. up to 2·0 c.c., as a rule, no precipitate appeared, even after some hours. These large amounts of NaOH are, however, themselves toxic to the virus (Table IV).

Table IV.

To the stated volumes of *N*/1 NaOH solution in water, was added distilled water to bring the volume to 5 c.c. and after thorough mixing, 5 c.c. of active virus juice were added and well mixed. After 2 hours' contact at room temperature, the mixtures were inoculated to, in each case, six young tomato plants, with the following results:

0·5 c.c.	*N*/1 NaOH	100	% positive
1·0 c.c.	„	83	„
1·5 c.c.	„	16·6	„
2·0 c.c.	„	0	„
0	„	100	„

In order, therefore, to prevent precipitation by the alcohol one had to use a quantity of NaOH which already of itself reduced the infectivity of the virus. It seemed, however, that this might make the test of the action of alcohol still more sensitive, and the following experiment was therefore carried out.

To three test-tubes were added 1·3 c.c., 1·6 c.c., and 2·0 c.c. respectively of normal NaOH solution. To these were then added absolute alcohol sufficient to bring the final mixture to 60 per cent., and after mixing well, 5 c.c. virus juice were added, and thoroughly mixed. All the tubes remained free from visible precipitate (and remained clear for 8 hours at least).

Four tubes were then taken, *A*, *B*, *C*, *D*. To *A* were added 1·3 c.c. *N*/1 NaOH solution, then 9·45 c.c. absolute alcohol, and these mixed well; then 5 c.c. filtered virus juice were added and thoroughly mixed. After standing at room temperature for 1 hour 50 minutes, during which time no precipitate appeared, the mixture was inoculated to 8 young

Kondine Red plants in the usual way. Of these, two, viz. 25 per cent., developed the disease, both after an incubation period unusually prolonged, viz. 18 and 21 days respectively.

To *B* was added no NaOH, but 1·3 c.c. water instead, and then alcohol and juice as before. After 1 hour's contact, the mixture was centrifuged for 30 minutes, the supernatant liquid removed, and replaced by 5 c.c. water, in which the deposit was well shaken up. This was then inoculated to 8 plants as before, all of which developed the disease, 7 on the 9th day and 1 on the 13th day.

To *C* were added 1·3 c.c. *N*/1 NaOH, 9·45 c.c. water (*i.e.* no alcohol), and 5 c.c. virus as before. After 1 hour 45 minutes, the mixture was inoculated to 8 plants. Of these, two, *i.e.* 25 per cent., developed the disease, and again in both cases late, viz. on the 16th and 21st days. The alkali alone, therefore, without alcohol, reduced the infectivity of the virus to the same extent as did the alkali-alcohol mixture.

To *D* were added 10·75 c.c. water, and 5 c.c. virus juice; and after $1\frac{1}{2}$ hours the mixture was inoculated to 8 plants, all of which developed the disease by the 14th day, five of them in 9 days.

It appears then that the presence of alcohol to 60 per cent. in the alkalised mixture did not reduce still further the infectivity of the virus already lowered by the alkali alone, and there is nothing to suggest that in the case of this plant virus, alcohol in the concentration used is really toxic to the virus, and is able, if the formation of a protective precipitate is prevented, to exert its toxic action and destroy the virus.

Cultivation outside the Plant. Hitherto all recent attempts to obtain increase of any plant virus outside of living plant tissue have failed, with one exception. Olitsky(12), using no unusual or special technique, inoculated sterile normal tomato juice with the juice of mosaic tomato, subcultured from this into normal juice, and obtained infection even with the 12th subculture. This represented a dilution of $4/10^{16}$, far outside any possibly infective dilution of the original inoculum. Several workers have tried to repeat this experiment but without success (Goldsworthy(5), Mulvania(10), Purdy(14)), and no explanation of the difference in result is as yet available. Equal want of success has attended all experiments with the Aucuba or yellow type of mosaic, but, since the difference from Olitsky's result may be due to some apparently minor point of technique, one such experiment is given here in some detail in spite of its negative result.

Sterile tomato juice was obtained from young actively growing normal tomato plants by the same method as was used in the

preparation of filtered virus juice, the crude juice in this case being passed through filter-paper, instead of sand and paper-pulp, before being passed through the "L 1" and "L 3" candles. After final filtration it was distributed in 5 c.c. volumes and incubated for 1 week at 27° C. Successive batches of such juice were prepared from time to time, and no juice was used that was more than 3 weeks old. The *p*H of this normal juice in four successive batches was 5·5, 5·1, 5·5 and 5·5 tested colorimetrically. After incubation, tubes were inoculated in the following manner from young tomato plants showing recent and well-marked signs of the Aucuba disease. Petioles, or young stems, were cut across with a sterile scalpel, seared at the end with a red hot knife, capillary tubes inserted through the seared surface and small quantities of juice sucked up and transferred to tubes of the medium. In all, 21 tubes were so inoculated, constituting the 1st subculture, and were then incubated at 27° C. After 5 days, from each of these tubes 0·1 c.c. was transferred to a tube of fresh medium (a separate pipette being used for each tube); this constituted the 2nd subculture. The process was repeated every 5 days, giving the 5-day series of cultures. Similarly, every 10, 15 and 25 days, subcultures were made, giving the 10-day etc. series. There were therefore 4 series of subcultures, differing in the periods of incubation. Any tube showing obvious contamination was rejected, but this rarely occurred. From time to time tests of the subcultures were made by inoculation to plants. For this purpose, from each of all the tubes of a subculture in any one series 0·2 c.c. was withdrawn as a sample, the samples mixed together, and the mixture inoculated to young tomato plants in active growth. Inoculation was made by needle prick, at least 80 punctures per plant in 4 leaves, and further, a small pledget of cotton wool soaked in the juice was inserted into an incision in the stem. The plants were held for at least 5 weeks, and examined regularly. The batches of uninoculated medium were tested in the same way, always on 6 to 8 plants.

The results of all inoculations made up to the 4th subculture are shown in Table V. The amount of the original inoculum of infected material could not be exactly measured, and varied a little in every tube with the quantity of juice taken up in the capillary, which also took up small pieces of tissue. It was estimated to be about 0·01, and not to exceed 0·05 c.c. in any tube. Taking the larger figure as an outside estimate the dilution of the inoculum was in the 1st, 2nd, 3rd and 4th subcultures, 1×10^2, 5×10^3, 25×10^4 and 125×10^5 respectively. The 3rd subculture, then, represents a dilution of the original inoculum of

Table V.

Series (days)	Subculture	No. of plants	No. positive	% positive
5	1st	4	2	50
	2nd	6	1	16·6
	3rd	8	1	12·5
	4th	10	0	0
10	3rd	6	2	33·3*
	4th	8	0	0
15	4th	8	0	0
25	4th	8	0	0

* This figure is unreliable, since the test of the batch of medium used for this subculture gave one positive result.

1 in 250,000, a dilution still possibly infective: but the 4th subculture, being a dilution of 1 in 12,000,000, is outside the range of still infective dilution. As is shown in Table V, in no case was infection got with the 4th subculture (nor in several examinations of later subcultures); but in one at least of the series the 3rd subculture was still slightly infective. No evidence, therefore, was obtained of multiplication of the virus.

This experiment was repeated the next season, using as normal medium a juice differently prepared. Here the tissue was ground up in an apparatus devised by W. A. Roach(15), and so finely that no intact cells could be detected under the microscope; the liquid was then passed through a "L 1" and then a "L 3" candle. The preceding experiment was then repeated, using only one series, viz. 7 days' incubation, and the original inoculum consisting of 0·1 c.c. of filtered virus juice, which was proved to be very active. Eight plants were inoculated with each subculture: the 1st subculture gave 7 positive, the 2nd 2, the 3rd, 4th and 5th, none.

It was again repeated in 1927 in this laboratory by Dr H. H. Storey on the lines of the first experiment described above, using a 7–8 days series, and inoculating with active filtered juice. In this also no evidence of multiplication was obtained. A number of modifications of different kinds has been tried, but all have failed.

Our experience, therefore, has been the same as that of all other workers, with the exception of Olitsky. There is, however, a possible explanation of our failure, which has not, so far as we know, been tested in the case of plant viruses. It may be, that for successful inoculation two factors are necessary, the virus itself and an accessory, non-multiplying factor, of which neither alone is sufficient to produce the disease but the two together are capable of causing infection. This, according to Gye(6), is true of the filterable chicken sarcomas of Rous.

It might, therefore, be the case that growth of the virus did occur in our subcultures, but its presence was not detected on inoculation owing to the loss of the accessory factor either through the high dilution involved in the subculturing or through ageing or deterioration. We have not so far succeeded in demonstrating the existence of such an accessory factor, and only one experiment, carried out here by Dr H. H. Storey, will be mentioned. Virus juice was precipitated by 60 per cent. alcohol, and the supernatant liquid filtered. This supernatant liquid is itself, as has been shown above (p. 160), incapable of producing the disease, but, when it was added to the 4th subculture of virus in normal juice, did the mixture become active, either with or without preliminary evaporation of the alcohol from the supernatant liquid.

I have pleasure in thanking Miss M. M. Browne for her assistance in the growth and care of the many plants required in these experiments.

Summary.

A description is given of a mosaic disease produced in tomato by a virus, possibly identical with Johnson's Tobacco Virus 6, which differs from that of ordinary tomato mosaic in the brilliance and intensity of its leaf-symptoms, but in other respects is indistinguishable from it by the characters investigated.

The filtered juice of infected plants transmits the disease in dilutions in water up to 1 in 10,000, retains its activity for a year or more at room temperature, and withstands heating for 10 minutes at 80° C. but is inactivated at 90° C.

It is not inactivated by alcohol up to 90 per cent. The virus comes down with the precipitate, and is not destroyed when the formation of precipitate is prevented by the addition of NaOH.

Attempts at cultivation of the virus outside the living plant are described, all were unsuccessful. The methods employed in filtration, inoculation, etc. are given in detail.

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Fig. 1.

Fig. 2.

HENDERSON SMITH.—Experiments with a Mosaic Disease of Tomato (pp. 155-167).

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DESCRIPTION OF PLATE IX.

Fig. 1. Typical leaves of tomato infected with Aucuba or yellow mosaic.
Fig. 2. Leaf of tomato similarly infected, showing yellowing of the veins.
Photographs taken by V. Stansfield.

(*Received December 12th*, 1927.)

THE TOXICITY OF CERTAIN SULPHUR COMPOUNDS TO *SYNCHYTRIUM ENDOBIOTICUM*, THE FUNGUS CAUSING WART DISEASE OF POTATOES

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(With 8 Text-figures.)

CONTENTS.

Introduction.

Earlier experiments have shown that the toxic action of sulphur on the winter sporangia of *Synchytrium endobioticum* in soil varies considerably under different conditions both of season and of soil type (9, 10). There is evidence from the Rothamsted field (9, 10) and pot experiments (3) and from the pot experiments of Weiss (11) which suggests that

high soil acidity such as may arise from the oxidation of sulphur to sulphuric acid may alone be sufficient to kill the fungus, but the degree of acidity[1] required is too great for fertility. Certain of the results obtained (9, 3), however, suggest that sulphur has a second mode of toxic action which is effective at much lower acidities under certain unknown conditions. Researches dealing with the oxidation of sulphur in soil[2], considered in conjunction with purely chemical investigations on inorganic sulphur compounds[3], suggest that a variety of compounds may be formed during the course of the oxidation of sulphur to sulphuric acid, and it is possible that one or more of these may be responsible for this second toxic action. If some form of sulphur is ever to serve as a practical means of controlling wart disease in the soil it is more likely to be by means of this second type of toxic action than by raising the soil acidity. It was decided, therefore, to attack the problem of the variability of the action of sulphur as a soil fungicide by determining which of the compounds, at all likely to be formed when sulphur is added to soil, are toxic to the fungus, as a preliminary to determining the conditions under which such a compound might be formed in the soil. Since those compounds which are more toxic than sulphuric acid are the most likely ones to contribute to the solution of the problem, sulphuric acid was taken as the standard with which to compare the toxicities of the other compounds.

Experimental.

A. *Chemical.* The compounds that have been tested are arranged schematically in Fig. 1 in order of the degree of oxidation of sulphur and are placed as far as possible under the oxides from which they may be considered to be derived. The formulae of compounds, the existence of which has not been proved, are placed within square brackets. All the compounds were tested for purity, whether they had been prepared specially for the work or had been obtained already prepared (see Appendix I).

B. *Biological.* Winter sporangia of the fungus were obtained fairly free from other organic matter by removing the outer parts of ripe decaying warts, pressing them through fine muslin and centrifuging in

[1] About *p*H 3·4 according to the Rothamsted experiments, and *p*H 3·9 according to Weiss (11).

[2] Of especial interest are the papers of Guittonneau (5, 6) and Guittonneau and Keiling (7), the first of which gives a key to the extensive literature on the subject.

[3] The present position of our knowledge of these compounds is summarised by Bassett and Durrant (2).

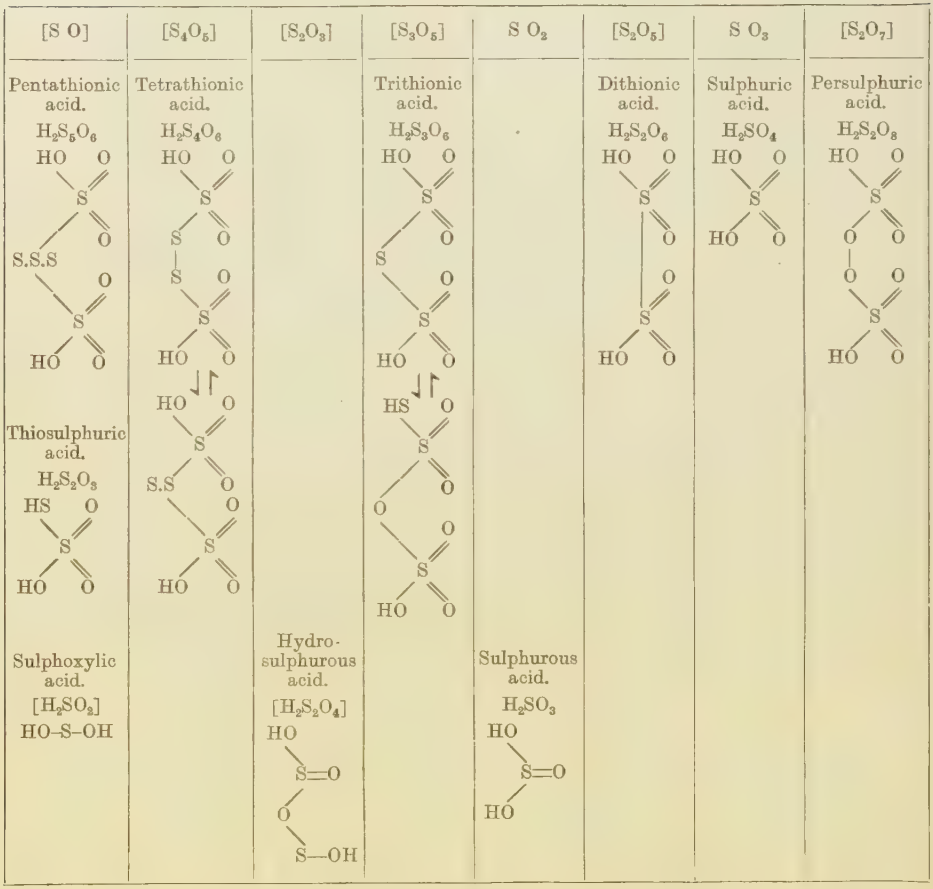


Fig. 1. Formulae of compounds tested, arranged according to the state of oxidation of the sulphur.

water 15 times for 25 seconds. Portions about 3 mm. in diameter of the damp sporangial material containing large numbers of sporangia were treated with 3 c.c. of the solution, the toxicity of which was to be estimated. After the required periods of exposure, usually 24 hours and 10 days, the sporangia were well washed and their viability tested. As they cannot yet be made to germinate in sufficient numbers in a reasonably short period of time, an indirect method of testing their viability, and so of estimating the toxicity of the compounds, was used. This depends on the differential staining in an aqueous solution of acid fuchsin

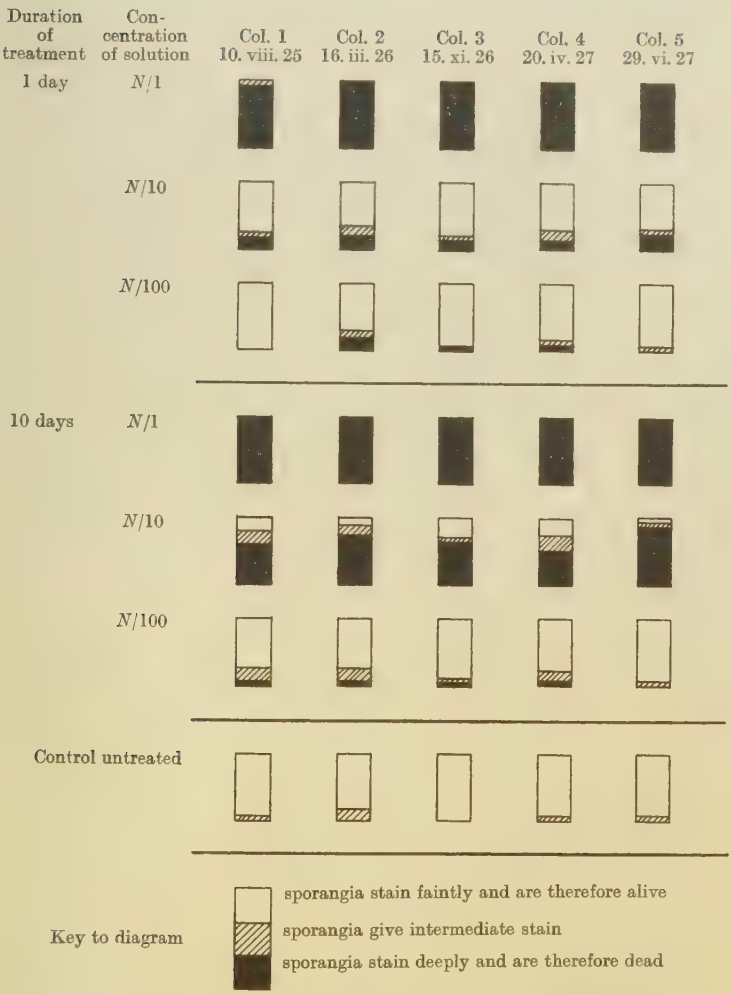


Fig. 2. Variation in toxicity observed at different times with different samples of sporangia treated with sulphuric acid.

of the contents of dead and living sporangia and has been described in a previous paper(4), where evidence of the reliability of the method is brought forward. The sporangia were mounted in 2 per cent. aqueous acid fuchsin under a coverslip which was gently pressed so as to expel the sporangial contents while under microscopic observation. Three counts of 20 sporangia were made for each test, and the numbers were recorded of (1) those which stained rapidly and deeply, as do dead sporangia, (2) those which stained faintly and slowly like living sporangia, or (3) those which were regarded as intermediate. The numbers of sporangia falling into each of these groups after treatment with the different sulphur compounds gave a measure of their toxicity.

Comparison of Toxicities.

Standard for comparison. Sulphuric acid.

Sulphuric acid has been taken as the standard with which to compare the toxicities of all the other compounds tested. A comparison of the results obtained from five similar tests with sulphuric acid, carried out at different times over a period of nearly two years, is shown in Fig. 2. The degree of variation shown includes that existing between different samples of sporangia at different times, together with any subjective observational variation[1] in placing the line of demarcation between the three groups, a process which requires some experience. A test with sulphuric acid was always carried out with each new batch of sporangia.

Sulphuric acid at the end of one day is completely toxic in normal solution and only slightly so in decinormal. At the end of 10 days about three-quarters of the sporangia are killed in decinormal solution.

Sulphuric, Dithionic and Sulphurous Acids, and their Neutral Alkali Salts.

The neutral alkali salts of sulphuric, dithionic and sulphurous acids exerted little, if any, toxic action, suggesting that the $'Na$, $'K$, $''SO_4$, $''S_2O_6$ and $''SO_3$ ions into which these salts are dissociated are non-toxic in neutral solution (Fig. 3).

The acids themselves were completely toxic (*i.e.* all the sporangia dead) in 10 days in normal[2], and partially so in decinormal solution (Fig. 3). The toxicities of these three acids were approximately equal when compared at the same normality. This coincidence suggests that

[1] The viability tests were all carried out by one worker, *i.e.* M. D. Glynne.

[2] Sulphur dioxide is insufficiently soluble in water for it to be possible to make up a normal solution, so only decinormal and more dilute solutions were tested.

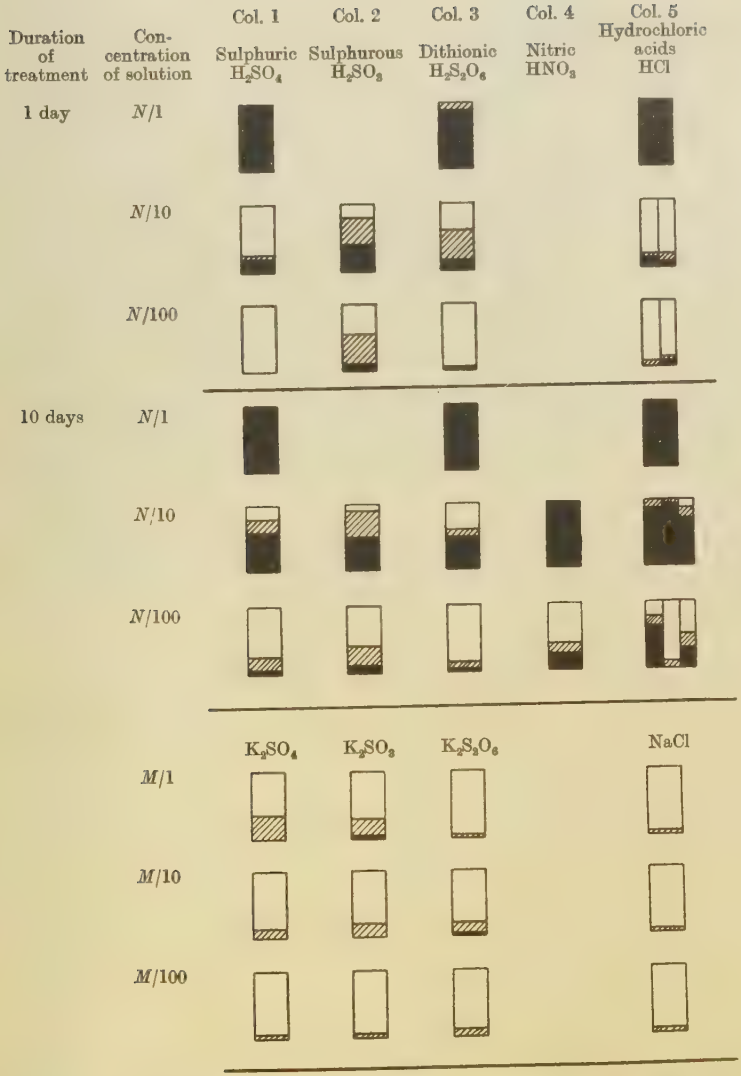


Fig. 3. Toxicities of sulphuric, dithionic, sulphurous, hydrochloric and nitric acids and their salts. (Three different observations are indicated for hydrochloric acid 10 days and two for hydrochloric acid one day.)

the $''SO_4$, $''S_2O_6$ and $''SO_3$ ions are also non-toxic in acid solution and that the equal hydrogen-ion concentrations of solutions of the same normality is the cause of their equal toxicities, in other words, that these three acids owe their toxicities mainly to their hydrogen-ion concentrations.

If this deduction be valid, then no acid should be less toxic than sulphuric when compared at the same hydrogen-ion concentration, and if an acid is of greater toxicity than sulphuric acid it would suggest either that its anion is toxic or that a toxic impurity is present in the solution.

For instance, none of the samples of hydrochloric or nitric acids tested had a toxicity less than that of sulphuric acid. (The toxicity figures obtained for hydrochloric acid at different times varied to an unusual degree but the lowest value is probably nearest the truth for the pure acid. The greater toxicity of the other two is probably due to the small amounts of free chlorine which so often occur in the concentrated acid.)

The conclusion is further strengthened by the fact that no acid tested in the course of this work was less toxic than sulphuric acid and by the fact that trithionic, tetrathionic and pentathionic acids were found to be of the same degree of toxicity as sulphuric acid (see Fig. 4, p. 176).

If the above acids owe their toxicities mainly to their hydrogen-ion concentration, then it follows that high acidity alone can kill the sporangia. The *p*H value of *N*/10 sulphuric acid, which in 10 days does not kill all the sporangia, is about 2, a value far below any found in fertile soil; even the value 3 for *N*/100 acid, which is of very low toxicity, is too low for fertility in soil and is definitely lower than the critical value found in pot experiments, viz. 3·4. Possibly this enhanced toxicity in the soil is due to some indirect effect of the acidity on the soil such as the liberation of toxic salts, *e.g.* those of manganese.

It has however been shown that the total effect of soil acidity does not account for the toxicity of sulphur under all conditions, so that another cause remains to be found.

Polythionic Acids.

Evidence had been obtained by one of us[1] that pentathionate is formed in soils to which sulphur has been added. The polythionic acids, in particular pentathionic acid, have been suggested by Young (12)

[1] W. A. Roach.

as the cause of the general fungicidal action of sulphur. The toxicities of these compounds are therefore of special interest.

The sulphur content of equimolecular solutions of sulphuric, trithionic, tetrathionic and pentathionic acids varies in the ratio of 1 : 3 : 4 : 5. In comparing the toxicity of sulphur in different chemical combinations it is necessary to test solutions containing equal quantities of sulphur. As the hydrogen ion has been shown to be toxic, all these solutions must also have the same hydrogen-ion concentration. These two ends are attained by adding to sulphuric acid of the requisite concentration a sufficient quantity of a neutral salt of the acid to be tested to supply a quantity of sulphur equal to that already contained in the sulphuric acid.

To take the polythionic acids as an example, to 1 litre of normal sulphuric acid is added $\frac{1}{6}$ gm. molecule of barium trithionate $Ba_2S_3O_6$, or $\frac{1}{8}$ gm. molecule barium tetrathionate $Ba_2S_4O_6$, or $\frac{1}{10}$ gm. molecule barium tetrathionate BaS_5O_6 respectively. The barium in each solution is precipitated, taking with it an equivalent amount of sulphate, but the normality of the solutions in respect to total acidity is unaltered. Thus the normalities of the three solutions are $2\,N/3$ (in regard to H_2SO_4) + $N/3$ (in regard to $H_2S_3O_6$), $3\,N/4$ (in regard to H_2SO_4) + $N/4$ (in regard to $H_2S_4O_6$), and $4\,N/5$ (in regard to H_2SO_4) + $N/5$ (in regard to $H_2S_5O_6$), respectively. Since all of these acids are strong ones their hydrogen-ion concentrations will not vary sufficiently in the above series to cause any variation in toxicity detectable by the method employed, so that these solutions have approximately the same hydrogen-ion concentration and contain equal quantities of sulphur in the various chemical combinations. They vary in their contents of $''SO_4$ ions but evidence that these are non-toxic has been brought forward.

Such solutions are all normal in regard to acidity and so may be designated $N/1$, but the symbol $'H/1$, representing as it does 1 gm. equivalent of hydrogen ions per litre, is perhaps more suited to the present purpose. They all contain $\frac{1}{2}$ gm. atom of sulphur per litre and so are conveniently represented in this respect by the symbol $S/2$; combining these two symbols we have $'H/1$, $S/2$. When the above solutions are 10 times diluted they will be represented by the combined symbol $'H/10$, $S/20$ and when diluted 10 times again $'H/100$, $S/200$, and so on.

The three polythionic acids themselves were of the same order of toxicity as sulphuric acid (Fig. 4, cols. 1, 2, 5, 7). Neutral solutions of their alkali salts (Fig. 4, cols. 4, 6, 8) were non-toxic. Sodium trithionate solution as tested in the first instance was almost completely toxic in 10 days in $S/2$ solution and slightly toxic in $S/20$ solution (col. 3). The solution became slightly acid on standing; this toxicity however was not found when the solution was carefully kept neutral by adding

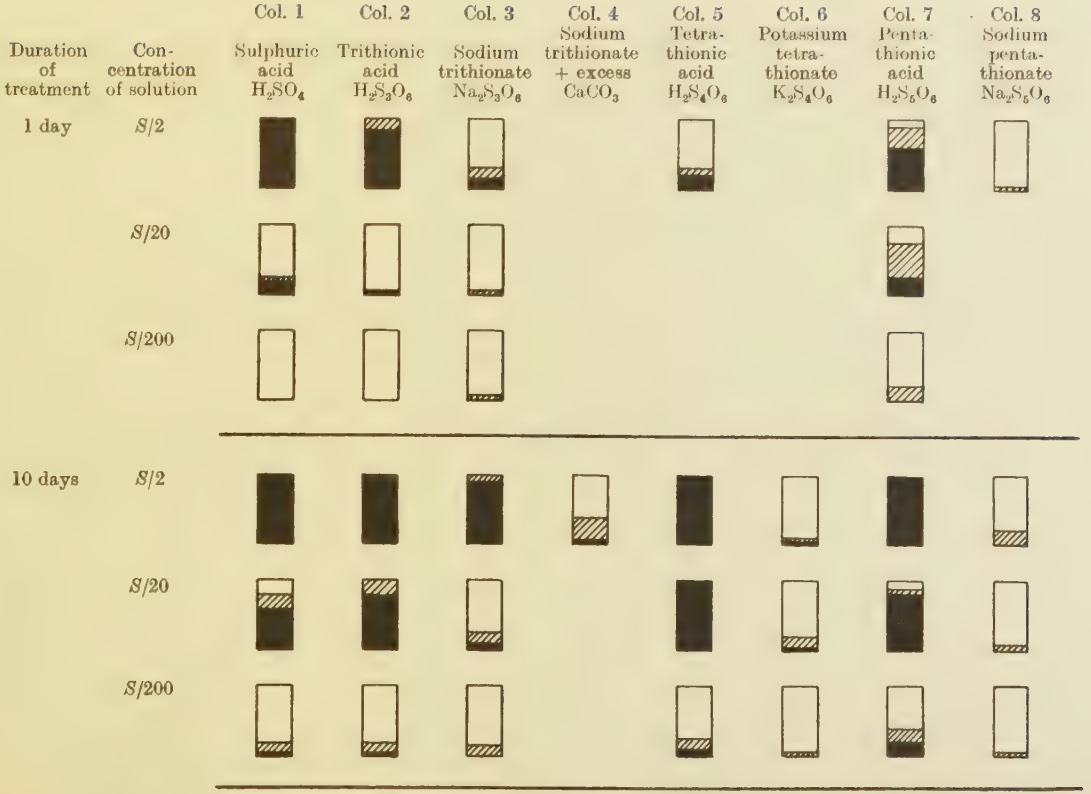


Fig. 4. Toxicities of polythionic acids and their salts compared with that of sulphuric acid.

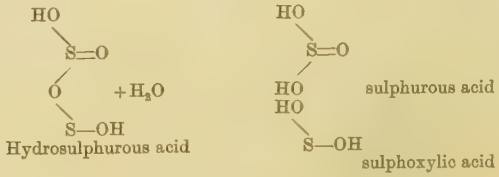
excess of calcium carbonate and by driving off sulphur dioxide by means of a slow stream of carbon dioxide (col. 4). The toxicity of the faintly acid solution will be referred to later (p. 182).

As none of the three polythionic acids is much more toxic than sulphuric acid, they do not appear to play an important part in the fungicidal action of sulphur towards *Synchytrium endobioticum*.

Thiosulphuric Acid, Thiosulphate, etc.

Sodium thiosulphate itself has no appreciable toxicity (Fig. 5, col. 3); but when it is acidified with sulphuric acid it has a high toxicity which shows itself with unusual rapidity, as indicated by the results obtained after treatment for one day only (Fig. 5, col. 2). Thiosulphuric acid is unstable except in dilute solution, as is shown by the fact that both the *N*/2 and the *N*/20 solutions rapidly deposit sulphur and give off sulphur dioxide. The toxicity of the solution might therefore be due either to the products of decomposition or to the undecomposed thiosulphuric acid. Sulphurous acid and polythionic acids, which are known to be products of the decomposition, are insufficiently toxic (Fig. 3, col. 2, Fig. 4, cols. 2, 5, 7) to account for the high toxicity of acidified thiosulphate solution.

As acidified thiosulphate solutions have powerful reducing properties, toxicity tests were carried out with sodium hydrosulphite $Na_2S_2O_4$, also a powerful reducing agent, which may possibly be formed in the decomposition of thiosulphuric acid. These were carried out both in neutral and in acid solutions. The solution of sodium hydrosulphite, which gave at first a slight but increasingly acid reaction and smelt strongly of sulphur dioxide, was definitely toxic (Fig. 5, col. 5). A neutral solution of the salt was obtained by adding calcium carbonate and passing a stream of carbon dioxide through the solution to remove the sulphur dioxide formed. Under these conditions the toxicity was negligible (Fig. 5, col. 6). When sulphuric acid was added to sodium hydrosulphite the resulting solution showed the same high order of toxicity as acidified thiosulphate (Fig. 5, col. 4). Now hydrosulphurous acid $H_2S_2O_4$ is a mixed anhydride of sulphurous and sulphoxylic acids; as may be seen from the following formulae:



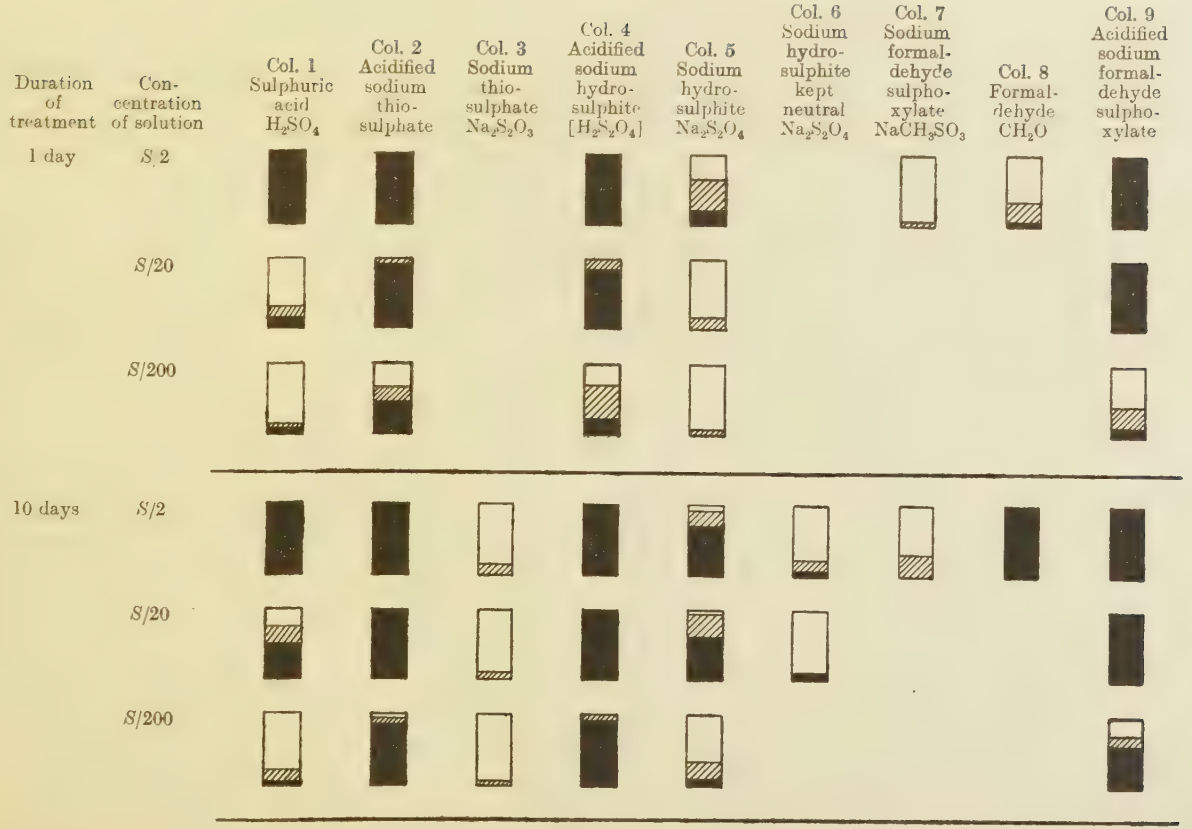
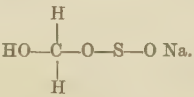

Duration of treatment
Concentration of solution
Col. 1 Sulphuric acid H_2SO_4
Col. 2 Acidified sodium thiosulphate
Col. 3 Sodium thiosulphate $Na_2S_2O_3$
Col. 4 Acidified sodium hydrosulphite $[H_2S_2O_4]$
Col. 5 Sodium hydrosulphite $Na_2S_2O_4$
Col. 6 Sodium hydrosulphite kept neutral $Na_2S_2O_4$
Col. 7 Sodium formaldehyde sulphoxylate $NaCH_2SO_3$
Col. 8 Formaldehyde CH_2O
Col. 9 Acidified sodium formaldehyde sulphoxylate
1 day
S/2
S/20
S/200
10 days
S/2
S/20
S/200

Fig. 5.

In solution it behaves as a mixture of these two acids. It has been shown that sulphurous acid (Fig. 3, col. 2) is not highly toxic, so sulphoxylic acid remains as the possible toxic agent. Neither free sulphoxylic acid nor its sodium salt are known to exist but the sodium salt is known in combination with formaldehyde as the compound sodium formaldehyde sylphoxylate.



This substance was found to be non-toxic in neutral solution (Fig. 5, col. 7). (The fact that it is less toxic than the formaldehyde which it "contains" is probably due to the formaldehyde suffering a molecular rearrangement on combination with the sodium sulphoxylate. That such a rearrangement does take place has been established by chemical means.) In acid solution (Fig. 5, col. 9) however it develops approximately the same degree of toxicity as acidified thiosulphate. Thus the three very unstable solutions obtained by liberating thiosulphuric, hydrosulphurous and sulphoxylic acids respectively from their salts by the addition of sulphuric acid are of the same high order of toxicity. From the work of Bassett and Durrant(2) and others cited by them it is obvious that these three acids are very closely interrelated, so that it seems possible that the toxicity of all three solutions may be due to the same substance formed from all three acids. All three solutions contain a variety of compounds but they are definitely known to contain thiosulphuric acid. It is possible therefore that the toxicity of all three solutions may be due to free thiosulphuric acid or some compound closely related to it, such, for instance, as Bassett and Durrant's postulated anhydro-acid.

An attempt was made to discover whether the toxicity of acidified thiosulphate solutions is due to some transitory compound formed as an intermediate product in the decomposition of the liberated thiosulphuric acid or to some compound contained in the more or less balanced solution which is known to be obtained a few hours after the acidification. The instability of many of the sulphur compounds formed and the length of exposure of the sporangia necessary for any measurable toxicity to show itself, constitute serious difficulties in determining the toxicities at all accurately. The following experiments, however, were carried out: a stock solution of *H*/10, *S*/20 acidified thiosulphate was made up and its toxicity was tested periodically. Samples of the clear

liquid, as free from precipitated sulphur as possible[1], were withdrawn at the end of 0, 1, 2, 4, 6, 8 hours, 1, 2, 4 and 7 days respectively. Sporangia were then treated with each sample for 24 hours. The toxicity figures obtained were constant within the limits of experimental error. Samples of an $H/100$, $S/200$ thiosulphuric acid solution were tested immediately after the solution was made up, at the end of 1 day and at the end of 7 days. The toxicity of all three samples was approximately the same.

If the toxicity is due to a transitory intermediate compound it should decrease after the disappearance of the compound sometime after the solution is made up. Since the toxicity does not decrease appreciably in 7 days it cannot be due to such a transitory intermediate compound but to a constituent of the balanced solution.

To determine which constituent of the balanced solution is responsible for the toxicity is of even greater difficulty and uncertainty. The chemical work done on acidified thiosulphate by Bassett and Durrant (2) and others, taken in conjunction with the facts already recorded in this paper, point to thiosulphuric acid itself or some compound closely related to it as the most likely toxic substance of those known to be present in the solution, viz. thiosulphuric acid (in small quantity), tri-, tetra-, and penta-thionic acids, sulphurous acid, sulphuric acid, sulphur (except in dilute solutions), etc. A further test was devised to give evidence on this question.

Since sulphurous acid is a decomposition product of thiosulphuric acid, the replacement of sulphuric acid by sulphurous acid in making up the solution will tend to produce a greater concentration of thiosulphuric acid in the equilibrium mixture obtained without appreciably affecting its acidity. A comparison of the toxicities of corresponding members of the two series was therefore made. (For details *re* making of these solutions see Appendix II, p. 189.)

As sulphuric and sulphurous acids in equal concentrations have about the same toxicity, any difference in toxicity found between members of a pair in the two series may be attributed to some indirect effect on the equilibrium mixture. The results (Fig. 6) show on the whole a slightly greater toxicity in the solutions in which sulphurous acid is in excess, but the differences are too small and irregular to warrant a definite conclusion.

[1] The liquid was not filtered because in doing so sulphur dioxide would be lost by evaporation and oxygen would be absorbed; both of these changes are likely to cause changes in the amounts of the other compounds in the balanced solution.

The precipitation of sulphur in the solutions after they had been allowed to stand overnight, *i.e.* 12 hours, is indicated in Fig. 6. An *S*/500 "excess sulphuric acid" solution, which was very slightly cloudy, corresponded with an "excess sulphurous acid" solution of concentration between *S*/200 (clear) and *S*/100 (cloudy), so that if the toxicity were due

Concentration of solution	Amount sulphur precipitated	Appearance of solution	Excess sulphuric acid	Excess sulphurous acid	Amount sulphur precipitated	Appearance of solution
S/20	much				little	
S/50	little	cloudy			none	cloudy
S/100	none	cloudy			none	cloudy
S/200	none	cloudy			none	clear
S/500	none	very slightly cloudy			none	clear
S/1000	none	clear			none	clear
S/2000	none	clear			none	clear

Fig. 6.

to the separated sulphur then the excess sulphurous acid solutions should be between two and a half and five times as toxic as the excess sulphuric acid. The fact that the toxicities do not correspond with the degree of separation of sulphur suggests that there is no connection between toxicity and colloidal sulphur or sulphur in a finely divided state.

Estimations of the amounts of thiosulphuric acid in the various solutions hitherto have given unreliable results so that it has not yet been possible to establish a quantitative relationship between thiosulphuric acid and toxicity. The qualitative evidence obtained, however, appears to justify the tentative conclusion that in solutions of the sulphur compounds considered, which are more toxic than sulphuric acid at the same hydrogen ion concentration, the excess toxicity is due to thiosulphuric acid, or some compound closely related to it, and formed from it on acidification.

On this theory the previously unexplained toxicity of trithionate solution becomes clear (pp. 176, 177). It becomes acid on standing, and in slightly acid solution it is known to decompose, giving rise to a certain amount of thiosulphuric acid. As the salt is non-toxic when its solution is kept neutral it seems probable that the toxicity of the solution of the salt which is not kept neutral is due to the thiosulphuric acid which is produced. Whereas both *S*/2 and *S*/20 solutions of sodium trithionate decolorised definite amounts of iodine after standing 10 days, solutions of trithionic acid, which was not more toxic than sulphuric acid, decolorised no iodine, showing that no thiosulphuric acid had been formed. Solutions of dithionic, tetrathionic and pentathionic acids and of their sodium salts also had no definite iodine value after standing 10 days.

Hence all the facts so far considered support the conclusions: first that the toxicities of all the sulphur acids so far considered which do not give thiosulphuric acid as a decomposition product are conditioned by their hydrogen ion concentrations; and secondly, when thiosulphuric acid is formed it bestows on the solution a greatly enhanced toxicity.

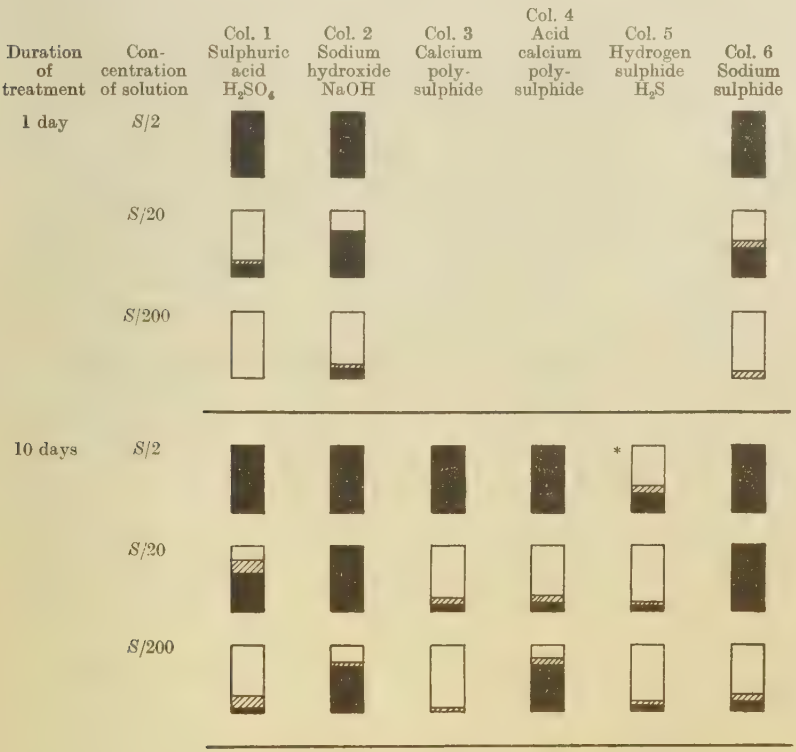
Sulphides and Polysulphides.

Sulphides and polysulphides cannot be looked upon as intermediate products in the formation of sulphuric acid from sulphur, but they are included because of the possibility of their formation either from sulphur itself or from the intermediate products so far considered.

Though sulphur does not appear to exist in a normal soil in the state of sulphide, sulphuretted hydrogen is so often a product of decomposition of most of the compounds so far investigated that it seemed necessary to test the toxicity of sulphur in this form.

Sulphuretted hydrogen. To make up each solution the appropriate quantities of pure sodium sulphide and sulphuric acid were shaken together until all the solid had dissolved. An *S*/2 solution could not be made up because sulphuretted hydrogen is insufficiently soluble, its

saturated solution at room temperature being approximately $S/8$. It is seen that sulphuretted hydrogen solution, or hydrosulphuric acid as this solution is sometimes called, has only a low degree of toxicity (Fig. 7, col. 5).



* Saturated solution.

Fig. 7. Toxicity of sulphides, polysulphides, etc., compared with that of sulphuric acid.

Sodium sulphide. $S/2$ sodium sulphide solution was prepared by dissolving the pure solid in water. The toxicity of the solution is probably explicable in terms of its alkalinity (Fig. 7, col. 6). *Sodium hydroxide* is seen to be more toxic than sulphuric acid when compared at equivalent concentrations.

Calcium polysulphide was prepared by the usual laboratory method. It is seen to be only slightly toxic (Fig. 7, col. 3) and such toxicity as it has may well be due to its alkalinity. In the "acid calcium polysulphide"

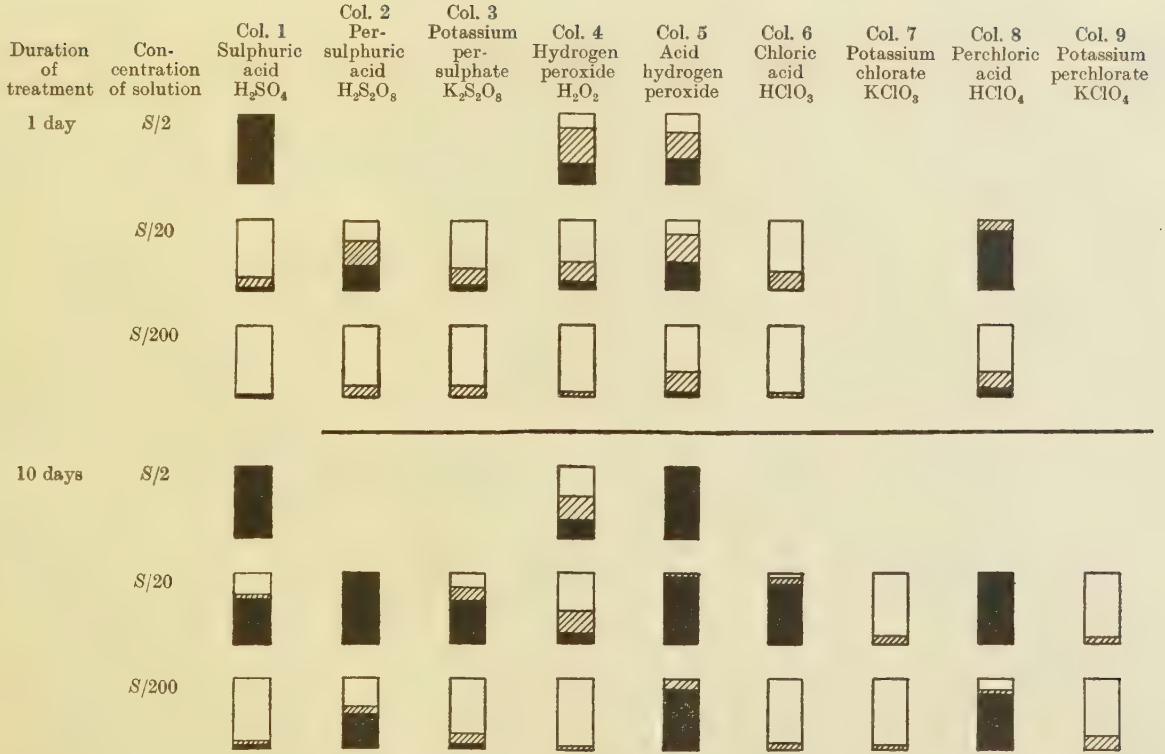


Fig. 8. Toxicity of oxidising agents.

the solutions were diluted with $N/100$ sulphuric acid instead of with water. The two more concentrated solutions still remained alkaline and their toxicities were little, if at all, affected by the addition of the small quantity of acid. The most dilute solution, however, was slightly acid and its toxicity was considerably increased by the addition of an amount of acid which alone had little toxicity (Fig. 7, cols. 3–4). The small quantity of thiosulphate which calcium polysulphide solutions almost invariably contain, which when acidified would set free thiosulphuric acid, was probably the cause of this increase in toxicity.

Sulphides and polysulphides do not appear to be sufficiently toxic to *Synchytrium endobioticum* to play any important part in the fungicidal action of sulphur, especially as not more than minute quantities of them ever occur in normal soils to which sulphur has been added; they were therefore not tested further.

Oxidising Agents.

It is known that the slow combustion of sulphur in the air gives rise to the formation of hydrogen peroxide. This in the presence of the sulphuric acid which also is a product of the slow oxidation of sulphur in air might well form persulphuric acid. In fact Barker, Gimingham and Wiltshire (1) observed that a potassium iodide starch paper held near warm moist sulphur soon became blue; a similar paper also turned blue when moistened with the liquid draining from the moist sulphur. The toxicities of hydrogen peroxide and persulphuric acid are therefore of interest although we have at present no evidence of the formation of either of these compounds in soil and sulphur mixtures.

Persulphuric acid and potassium persulphate. From Fig. 8, col. 2, it is seen that persulphuric acid has a high toxicity. After 10 days an $S/200$ solution, *i.e.* one to which 0·0016 per cent. sulphur was added in the form of persulphuric acid, was about as toxic as an $S/20$ solution of sulphuric acid, *i.e.* persulphuric acid is about ten times as toxic as sulphuric acid. Potassium persulphate solution also had a definite toxicity (Fig. 8, col. 3), which however was only about one-tenth that of the acid itself. By the end of the test the solution had become acid, so the compound was tested again in a solution kept neutral by being agitated gently with an excess of barium carbonate. Under these conditions its toxicity was small.

Hydrogen peroxide solutions of the same oxidising powers as the persulphate solutions were tested. Hydrogen peroxide in neutral solution was of low toxicity. In sulphuric acid solution it was highly toxic,

in all probability, owing to the formation of persulphuric acid (Fig. 8, cols. 4 and 5).

Chloric and perchloric acids were tested to see whether other strong oxidising agents were also toxic. Chloric acid was of approximately the same toxicity as sulphuric acid and perchloric acid about ten times as toxic and the neutral salts non-toxic (Fig. 8, cols. 6, 7, 8, 9). To follow the question further was considered outside the field of the present investigation though interesting problems suggest themselves.

Summary.

The toxicities towards the winter sporangia of *Synchytrium endobioticum* of certain of the simpler sulphur compounds which are at all likely to be formed when sulphur is added to soil were tested and compared with that of sulphuric acid.

Sulphuric (H_2SO_4), sulphurous (H_2SO_3), dithionic ($H_2S_2O_6$), trithionic ($H_2S_3O_6$), tetrathionic ($H_2S_4O_6$), and pentathionic ($H_2S_5O_6$) acids were toxic and this toxicity was of the same order in each case at the same hydrogen ion concentration. Their neutral salts were non-toxic. These facts suggest that the toxicities of these acids are mainly due to their hydrogen ion concentrations.

Acidified solutions of sodium thiosulphate $Na_2S_2O_3$, sodium hydrosulphite $Na_2S_2O_4$ and sodium formaldehyde sulphoxylate were about ten times as toxic as sulphuric acid.

Evidence is brought forward which suggests that the toxicity of these acidified solutions, in excess of that accounted for by the hydrogen ion concentration, is due to the thiosulphuric acid present in each of them. In view of the experimental difficulties due to the instability of some of the compounds and the length of time taken by them to exert their toxic action on the fungus investigated, this conclusion must be regarded as tentative.

Of the other compounds tested sodium hydroxide was found to be a little more toxic than sulphuric acid and persulphuric acid about ten times as toxic; hydrogen peroxide, calcium polysulphide and sulphuretted hydrogen were only slightly toxic.

APPENDIX I.

Notes on the Preparation, Purification and Analysis of the Polythionates.

The work described in the main part of the paper has been so dependent on the purity of certain of the compounds, especially the polythionates, that it seems desirable to append brief descriptions of the methods of preparation and purification of these compounds and analytical data establishing their degree of purity.

Sodium trithionate $Na_2S_3O_6$ was prepared by the method described by Plessy (8). It was purified until free from thiosulphate. The crystalline precipitate was dried over quick-lime *in vacuo* and kept in a bottle with a well ground-in stopper.

Analysis (11. xi. 26). The chlorine value determined in a Bunsen[1] oxidation apparatus was 94·7 per cent. of the value calculated for pure $Na_2S_3O_6$. 0·5 gm. substance dissolved in water decolorised 1 drop of $N/20$ iodine but not 2 drops; hence no more than a trace of sulphite or thiosulphate was present. A solution of the substance gave a precipitate with barium chloride. 0·5 gm. substance was dissolved in water and the sulphate precipitated by means of barium chloride in the cold. The precipitate was spun down on the centrifuge, the supernatant fluid being discarded. The precipitate was alternately suspended in distilled water and spun down until the discarded supernatant fluid no longer gave a precipitate with silver nitrate. The precipitate was then washed into a weighed crucible, dried, ignited and weighed, its weight being 0·0478 gm., which corresponds to 0·0291 gm. Na_2SO_4 or 5·8 per cent. Na_2SO_4 in the salt. This figure is likely to be in excess of the true one because of the known property of barium sulphate precipitates of taking down salts with them, especially in the cold; thus the figure is in sufficiently close agreement with the one determined by difference, *i.e.* $100 - 94{\cdot}7 = 5{\cdot}3$ per cent.

As an additional check the total sulphur in 0·5 gm. substance was determined by oxidation to sulphate and weighing as barium sulphate. Its weight was 1·4184 gm. Subtracting 0·0478 gm. *i.e.* the weight of $BaSO_4$ equivalent to 0·0291 gm. Na_2SO_4 we have left 1·3706 gm. $BaSO_4$ which corresponds to 0·4667 gm. $Na_2S_3O_6$ in the 0·5 gm. substance, *i.e.* 93·3 per cent. This figure is lower than the one calculated from the chlorine value, viz. 94·7; this fact no doubt is partially explained by the high value for the estimated Na_2SO_4 content.

The analysis of the sample was taken to be

$Na_2S_3O_6$	94·7 per cent.		$Na_2S_3O_6$ 95 per cent.
Na_2SO_4	5·3 ,,	in round figures	Na_2SO_4 5 ,,
$Na_2SO_3 + Na_2S_2O_3$	trace only		$''SO_3 + ''S_2O_3$ trace only.

To make up the solution of trithionic acid the requisite amount of $Na_2S_3O_6$ was added to sulphuric acid of the correct concentration. It had been ascertained that an excess of Na_2SO_4 had no effect on the toxicity of H_2SO_4, and therefore presumably it would have none on that of $H_2S_3O_6$ either.

Potassium tetrathionate $K_2S_4O_6$ was prepared by the method described by F. Raschig (Schwefel- und Stickstoffstudien (1924), ch. XXIII).

[1] The newer and more accurate method due to Treadwell and Mayr (*Z. anorg. u. allg. Chem.* XCII (1915), p. 127) had not at this time come to the notice of W. A. Roach who was responsible for the chemical part of this investigation.

Analysis (29. x. 26). Similar methods to those employed for the trithionate gave the following figures:

$K_2S_4O_6$ 99 per cent.
K_2SO_4 1 ,,
$K_2SO_3 + K_2S_2O_3$ trace only.

Barium pentathionate BaS_5O_6. Though newer methods were tried the following method, which is but a slight modification of the original one for the preparation of pentathionates, gave the purest sample of pentathionate obtained in the present investigation.

A Wackenroder solution was made in the usual way by alternately passing sulphuretted hydrogen into a saturated solution of sulphur dioxide until there was no further smell of sulphur dioxide and allowing to stand overnight, then passing in sulphuretted hydrogen again on the morrow when the smell of sulphur dioxide had reappeared. In this way a solution containing a mixture of the polythionic acids is obtained, in addition to much precipitated sulphur. As more sulphuretted hydrogen is passed in so the proportion of pentathionic acid increases and that of the lower polythionic acids decreases; finally the pentathionic acid disappears with formation of more sulphur. At the third attempt the passing in of sulphuretted hydrogen was discontinued when most of the soluble sulphur was in the form of pentathionic acid. The solution was filtered and evaporated on a water bath to about half its bulk and filtered again to free from precipitated sulphur. To the almost clear solution barium carbonate was added with much stirring. Much sulphur dioxide was expelled, sulphur and barium sulphate were precipitated. When excess of barium carbonate had been added and well stirred in the liquid it was filtered again. A clear filtrate was obtained. To this filtrate first alcohol, then ether was added to precipitate the barium pentathionate, which was collected and washed with alcohol on a Buchner funnel and dried in a vacuum desiccator over quick-lime. A further crop of salt was obtained from the filtrate by saturation with calcium chloride and purifying the precipitate by taking up in water and precipitating with alcohol and ether.

The powder dissolved readily in water giving a clear solution. The solution gave a dense precipitate of sulphur on the addition of concentrated caustic soda. It also gave the other tests for pentathionate. A solution containing 0·1 gm. substance did not decolorise 1 drop of $N/20$ iodine. It was therefore practically free from sulphite and thiosulphate.

Analysis. 0·5 gm. powder was boiled with 50 c.c. $N/10$ $KClO_3$ + 25 c.c. conc. HCl and the precipitated $BaSO_4$ filtered off, washed, ignited and weighed; 0·2656 gm. $BaSO_4$ obtained, corresponding to the barium in the powder. The $''SO_4$ in the filtrate was precipitated with barium chloride in the usual way and weighed.

0·2656 gm. $BaSO_4$ corresponding to Ba in powder.
1·3389 gm. $BaSO_4$ corresponding to $''SO_4$ in powder.

$$\text{Ratio Ba : S : : } \frac{0{\cdot}2656 \times \frac{137}{233}}{137} : 1{\cdot}3389 \times \frac{32}{233} : : 1 : 5{\cdot}041$$

Theory for BaS_5O_6 1 : 5·000

$$1{\cdot}3389 \text{ gm. } BaSO_4 = \frac{1{\cdot}3389 \times 393}{233 \times 5} = 0{\cdot}4517 \text{ gm. } BaS_5O_6$$

(Residue 0·0483 gm. water? BaS_5O_6, 2·3 H_2O)

Powder was 90·34 per cent. BaS_5O_6.

The chlorine value was 91·3 per cent. of the value calculated for BaS_5O_6. The value 90·34 calculated from the barium sulphate precipitate was accepted as probably more accurate than the value 91·3 calculated from the chlorine value.

S/2 solution. Since 0·5 gm. powder gave 1·3389 gm. $BaSO_4$ the powder contained $\frac{1{\cdot}3389 \times 100}{0{\cdot}5} \times \frac{32}{233} = 36{\cdot}78$ per cent. sulphur.

Therefore 25 c.c. *S*/2 solution contain $\frac{32}{2} \times \frac{25}{1000} = 0{\cdot}4$ gm. sulphur. 0·4 gm. sulphur is contained in 1·088 gm. salt.

Therefore to make up 25 c.c. $Na_2S_5O_6$ 1·088 gm. of the above sample of BaS_5O_6 were shaken with 25 c.c. *S*/2 Na_2SO_4 and the precipitated $BaSO_4$ removed by centrifuging.

To prepare $H_2S_5O_6$ *S*/2 H_2SO_4 was substituted for *S*/2 Na_2SO_4.

APPENDIX II.

Notes on the Preparation of the Solutions referred to on pages 180 and 181.

A graduated series of pairs of solutions was made up, one member of each pair having its *p*H adjusted by means of an excess of sulphuric acid and the other member having an equivalent amount of sulphurous acid instead. Each pair differed from the preceding pair by being of half the concentration. 25·8 gm. $Na_2S_2O_3$, $5H_2O$ were dissolved in water and the solution made up to 2000 c.c.; thus it was an *S*/10 $Na_2S_2O_3$ solution. 1000 c.c. of this solution were set aside in a Winchester quart bottle; 800 c.c. of the remainder were made up to 2000 c.c. with water to form an *S*/25 $Na_2S_2O_3$ solution. 1000 c.c. of this solution were set aside in a Winchester quart bottle. In this way were prepared a litre each of solutions of $Na_2S_2O_3$ of the following concentrations: *S*/10, *S*/25, *S*/50, *S*/100, *S* 250, *S*/500, *S*/1000, which were placed in a row in Winchester quart bottles. In a similar manner by starting with 24·8 gm. $Na_2S_2O_3$, $5H_2O$ + 25·2 gm. Na_2SO_3 for the first solution were prepared a series of solutions of the following concentrations: *S*/10 $Na_2S_2O_3$, *S*/20 Na_2SO_3; *S*/25 $Na_2S_2O_3$, *S*/50 Na_2SO_3; ... *S*/1000 $Na_2S_2O_3$, *S*/2000 Na_2SO_3. These solutions also were contained in Winchester quart bottles and placed in a row. By the side of each of these rows of bottles were placed bottles containing 1000 c.c. of sulphuric acid of the following concentrations: *H*/5, *H*/12·5, *H*/25, *H*/50, *H*/125, *H*/250, *H*/500. Finally the appropriate acid solution was added to the "$Na_2S_2O_3$" or "$Na_2S_2O_3 + Na_2SO_3$" solution as rapidly as possible and mixed thoroughly. In this way were obtained two series of solutions each of which consisted of solutions of the following concentrations of $H_2S_2O_3$; *S*/20, *S*/50, *S*/100, *S*/200, *S*/500, *S*/1000, *S*/2000. The acidity of these solutions in regard to $H_2S_2O_3$ was *H*/40, *H*/100, ... *H*/4000. In the first series there was an excess of sulphuric acid to make up the total acidities to *H*/10, *H*/25, ... *H*/1000; in the second series the excess of acid was in the form of sulphurous acid. There was also more sodium sulphate in the second series which, however, can be neglected as far as its toxic effect is concerned.

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ON *CLADOSPORIUM HERBARUM*: THE QUESTION OF ITS PARASITISM, AND ITS RELATION TO "THINNING OUT" AND "DEAF EARS" IN WHEAT

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(With Plates X and XI and 2 Text-figures.)

CONTENTS

Introduction.

In the autumn of 1923 the writer's attention was directed to a trouble in wheat crops in Yorkshire known locally as "thinning out" and "deaf ears." By "thinning out" the grower meant that a crop, which at the time of heading out looked most promising, became thinner from then until harvest owing to individual plants falling between the others. Such plants, and sometimes others which remained standing, assumed the colour of ripeness prematurely, but their ears contained either a few small, shrivelled grains, or none at all, and these ears were spoken of as "deaf ears" or "whiteheads." These conditions of the crop were reflected in a corresponding reduction in the yield of grain on threshing. The most serious case investigated at that time, where the trouble was

persistent, had during the preceding 10 years caused a monetary loss of some thousands of pounds. An experienced entomologist[1], who had observed the trouble on that farm for several years, stated that it was not due to insect or animal pests.

Such troubles in this country were attributed to (1) *Ophiobolus graminis* Sacc. (and possibly allied Ascomycetes), and (2) *Cladosporium herbarum* Lk. In some of the cases investigated Ophiobolus and similar fungi were completely absent, so that Cladosporium, which was generally present on some or all of the aerial parts of "deaf-eared" plants, was left as a possible cause. The literature dealing with this question, and in general use amongst agriculturists, being founded on the balance of experimental evidence, describes *C. herbarum* as a pathogenic organism. Mycologists, however, doubt this view, not because they have definite evidence against it, but because the records of the numerous investigators, on the whole, are confusing and contradictory. An outline of the records will throw light upon the present position.

Kühn (1876)[2] considered *C. herbarum* to be non-parasitic, "settling only on parts of plants diseased and dying as the result of other factors, *e.g.* unfavourable weather at the time of flowering, damage to the ears by late frosts, and animal or plant parasites" Laurent(6) held a similar view. Woronin (1891) apparently considered it to be a saprophyte, reporting that "it occurs along with many other fungi, which are favoured in their development by heavy rainfall." Janczewski(5), dealing with the occurrence of *C. herbarum* upon plants in periods of cold weather, and with the results of sowing grain bearing mycelium and conidia of the fungus, stated: "This and the experience of other observers shows without doubt that *C. herbarum* is merely an 'occasional-parasite,' capable of penetrating plants only when they are weakened by other conditions. Therefore, wilting leaves or over-ripe plants afford a favourable substratum for the fungus. The black growth occurs more especially on the ears, but the grains are not affected." Frank (1881) and Eriksson (1883) were also of opinion that *C. herbarum* was non-parasitic as a rule, but occasionally behaved as a parasite.

The opposite view, that *C. herbarum* is parasitic and pathogenic to cereals, is based mainly upon the works of Lo Priore(7) and Bancroft(1). Lo Priore investigated a disease of wheat grains which he termed "Nero dei cereali," in which brown streaks and black dots occurred about the top of the grain. His conclusions were that:—*C. herbarum* was the cause

[1] T. H. Taylor, Advisory Entomologist, University of Leeds.

[2] These references to earlier investigators are extracted from Lo Priore's paper (7).

of the trouble: the seedlings were attacked in their primary development and destroyed: the wheat plants were attacked at the lower portion of the haulm and, as a result of this, formed either none or only feeble ears: the ears were attacked at the time of flowering and formed no grains: the ears were attacked at the time of ripening...and the grains...developed peculiar black stripes[1]. Dealing later with another disease, termed briefly "puntatura" (the American "black point"), both Lo Priore(7) and D'Ippolito(3) stated *C. herbarum* to be the cause, but the recorded effects of the fungus appear to be strikingly contradictory to those previously reported. D'Ippolito stated: "Spotted grain...germinates regularly. In no case and in no stage of development of the plant was there any trace of mycelium." (This investigator favoured the "mycoplasm" theory.) An explanation for these conflicting results and conclusions is furnished by Peyronel(11), who states that these researches, in both cases, "conducted without aseptic precautions, without isolations or artificial cultures, and above all without controls, do not give certain assurance that *C. herbarum*...is the specific cause of puntatura." It should be added that Lo Priore recorded that his infected material (upon which his experimental conclusions and results were based) yielded *Dematium pullulans* de Bary (then believed to be a "liquid-conidial form" of *C. herbarum*), and *Alternaria tenuis*. Further, he identified the organisms present by placing diseased material in damp chambers on filter paper moistened with sugar solution; but these are the very conditions under which some parasitic fungi would not yield spores, and, therefore, would not be seen or identified (see p. 203). Thus, in addition to *C. herbarum*, there were present two other perfectly different fungi[2], and possibly others, consequently the results of the experiments could not be attributed with certainty to *C. herbarum*.

The work of Bancroft(1), carried out by more accurate methods, supported the conclusions of Lo Priore, and gave experimental evidence in favour of the view that *C. herbarum* is parasitic and pathogenic. This investigator stated that the life-cycle of this fungus comprised two phases: a Cladosporium phase in which the organism existed as a saprophyte, and a Hormodendron phase parasitic upon various kinds

[1] It is of interest to note that the symptoms recorded concerning the seedlings, diseased bases, feeble ears, and absence of grain, are similar to those in Fusarium disease (see p. 213 of this volume). Lo Priore mentions in his later work (7) on *C. herbarum*, an associated "mycelium (of) a rather intense rose colour"; as he did not identify this and apparently neglected it entirely, and did not work with pure cultures, it appears highly probable that Fusarium was present, and that this, not *C. herbarum*, caused the symptoms mentioned.

[2] Concerning *D. pullulans* de B. see Part 3 of this volume.

of plants. Hence the authority for the following references to *C. herbarum* on wheat plants: "Diseased grains, as a rule, do not germinate, but those that do grow produce diseased plants, which clearly show the mycelium of Cladosporium, under the form of long, reddish-brown specks, even in the first leaf-sheath (Massee(10))"; "Hormodendron spores are produced which spread the disease amongst living plants"; "It is believed that the disease may be one cause of 'deaf ears,' where no grain at all is formed in the ears." More recently Mackie(8) has stated that Sooty Mold (*Hormodendron cladosporioides*) each year causes serious losses in wheat fields in certain parts of U.S.A.

Under these circumstances the present writer would have felt justified in advising upon the practical problem of "thinning out" and "deaf ears" upon these lines, had he not been aware of the latent feeling of doubt amongst mycologists in general; further, one of them[1] stated that "no critical work concerning the pathogenicity of *C. herbarum* has been done in this country." In order to deal satisfactorily with the problem it was considered necessary to investigate *C. herbarum* with reference more particularly to:

(1) the forms of this fungus occurring upon cereals;

(2) the Hormodendron stage and its parasitism;

(3) the capacity of this fungus to cause "thinning out" and "deaf ears."

The Forms of *C. herbarum* occuring on Cereals.

The species *C. herbarum* Lk. is common everywhere on dead and decaying organic matter, and on a great number of different kinds of host plants. The following species of the genus Cladosporium are quoted from Rabenhorst(12) as occurring on cereals:

C. herbarum Lk.—universal on all plant and animal remains;

C. graminum Cda.—on the aerial parts of Gramineae;

C. atrum, *C. profusum*, *C. epiphyllum*—possible habitants of cereals.

In addition to these there are certain forms which may, or may not, belong to some of the foregoing species:

Hormodendron cladosporioides Bon.—belonging to *C. herbarum*;

H. herbarum Bon.—on plant stems;

H. hordei Bruhne—on living leaves of *Hordeum vulgare*.

It is now recognised that *C. herbarum* varies considerably in morphological and macroscopical characters under different natural conditions;

[1] A. D. Cotton, Pathological Laboratory, Ministry of Agriculture, Harpenden. 10. iii. 22.

such variations, described in somewhat different terms, may have given rise to the numerous specific names enumerated in the systematic works. Brooks and Hansford (2) have shown that the single species *C. herbarum* comprises numerous definite strains, the differences between which are comparable with the differences between so-called species; and they suggested the abolition of the names of several species and the inclusion of such forms as "strains of *C. herbarum*." For the purpose of the present investigation it was decided to ascertain whether the species listed above were true species or merely strains, and whether these species or strains differed in pathogenicity. This appeared to be an essential matter in the study of the relation of Cladosporium to the disease of "deaf ears," since any one of these organisms, or all of them, might be pathogenic to wheat.

Isolation and Differentiation of Strains.

Cladosporium was examined at various times of the year in its natural condition on different kinds of host plants, but most frequently on one or other of the four common cereals. It was found that the individuals showed differences not only on the different cereals, but also on the same kind of cereal; sometimes a single part of a plant showed apparently different kinds of the fungus simultaneously. The differences included colour of the organism in mass on the host, and when viewed on a slide in water under the microscope; length, width, septation, shape, colour and habit of conidiophores; and size and shape of conidia. It was impossible to relegate any of the individual natural specimens to a definite species, because as similar structural forms and variations occurred in all, there was no clear line of demarcation between them. Apparently different types of Cladosporium, as judged by macroscopic appearance, also appeared when naturally infected material which showed no fructification in the field was incubated at a constant temperature. Such apparently different types were carefully sought and utilised as the sources of pure cultures.

For the raising of cultures spore dilutions were prepared by transfers to successive drops of water, under aseptic conditions so far as practicable, and the final hanging drops which showed perfect separation of conidia, and total absence of any other kind of fungus, were used for dilution cultures in malt gelatine, poured at about 23° C. By transferring a small piece of mycelium from the edge of a colony[1] to each of the several kinds of medium used, a series of slant cultures was obtained

[1] The term "colony" is used for the growth on a plate from a single spore.

from one single conidium; and this, done in triplicate, afforded a means of noting any variation in the growth from that single spore on any of the media. Further, by dealing in this way with a number of colonies (usually six), comparison was possible between the growths from different single conidia from a given source. Amongst the media used were grape-juice gelatine, for comparison with Bancroft's investigation; beerwort gelatine, to compare with Mason's results(9); Dox's medium as used by Brooks and Hansford(2); further, synthetic media with dextrose, saccharose, and protein respectively served for comparison of the influence of different sources of carbonaceous food-material, and the sugar media and potato agar, at widely different hydrogen-ion concentrations, showed the influence of acidity and alkalinity; other media are referred to incidentally later. The object in view was to find, if possible, some means of distinguishing between the selected types of the fungus by their growth under controlled conditions.

Cultured according to the plan described, two facts were ascertained: (1) Types of *C. herbarum*, which differed from each other consistently, were obtained, but the differences between all the types were revealed only by the method of simultaneous culture on several media, not on any one medium. (2) A given type occurred on one kind of host more frequently than on other hosts, but no one form was confined to any one kind of host; what may be termed "prevalent" types were obtained from each of wheat, barley, oats, cabbage and broccoli. That these "prevalent" types differed consistently, and that the differences can be observed only by the use of several media, may be illustrated by reference to the macroscopic characters in culture as summarised:

Malt gelatine. As shown in Plate X, fig. 1, the types fall into two groups, (*a*) broccoli and barley, with well-developed aerial mycelium, and (*b*) cabbage, wheat and oat, with aerial growth so short and compact as to appear powdery; the wheat type eventually distinguished by its sepia colour[1].

Malt agar. Two groups as on malt gelatine, but here the types in the first group are distinguished from each other; the barley type gives aerial mycelium nearly white, abundant, and floccose, matting down to a thick, mainly olive-grey felt; the broccoli type gives aerial mycelium short and dense, passing through Lincoln green to dusky olive-green, with scattered whitish tufts, and matting down to a thin, dark greyish-olive felt. In the second group the wheat form is again characteristic.

Wheatmeal agar. As shown in Plate X, fig. 2, the members of the first group are distinguished from each other, owing to the superficial white growth of the barley type masking the olive-green growth below. In the second group the oat form is marked out by its distinctive colour.

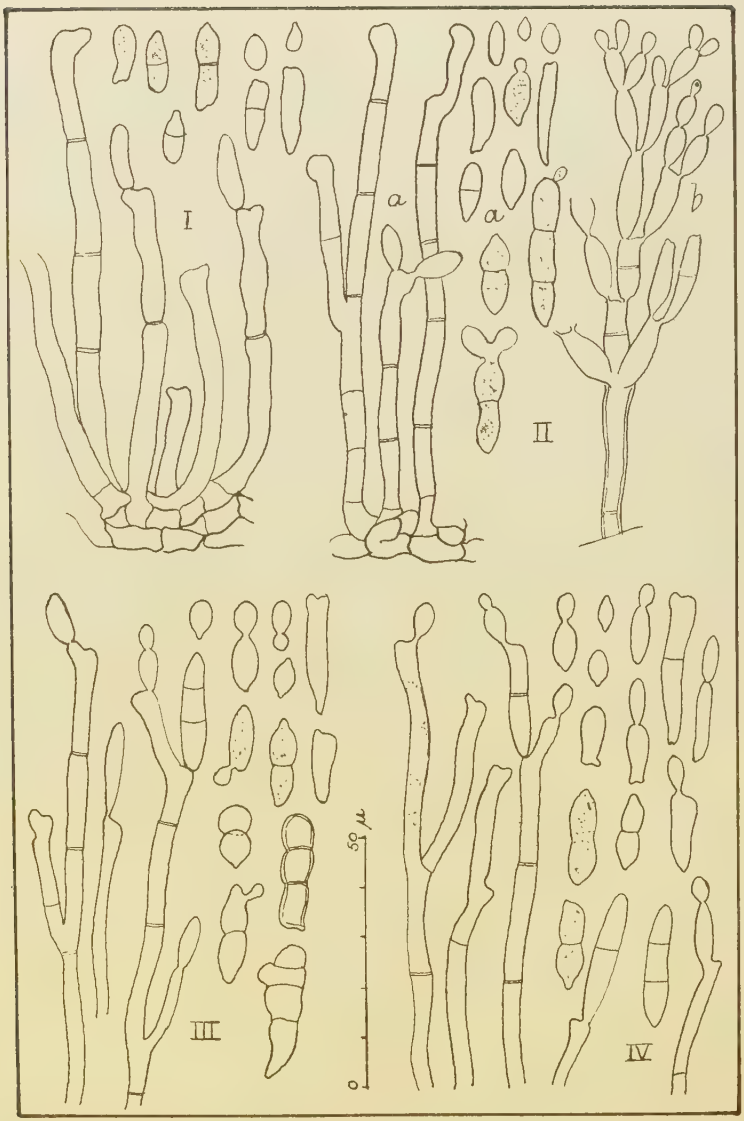
[1] Ridgway's "Color Standards and Nomenclature."

Dextrose and saccharose media. The two groups are clearly distinguished, but the distinctions between the members of each group are less definite than on the foregoing media. Growth is almost identical with the different sugars, and these give no help in rendering more prominent any type characteristics.

Hydrogen-ion concentration. Synthetic medium containing dextrose, adjusted to *p*H 5·5, 6·6 and 7·8, all other conditions being identical, was used. The results for the broccoli and wheat types are illustrated in Plate X, fig. 3, which shows that the macroscopic characters and approximate rate of growth (at 20° C.) were not distinctly modified; the other types behaved similarly. Difference of hydrogen-ion concentration to this extent, with both sugar and potato media, proved of no assistance in distinguishing more clearly between the types.

Since the above-mentioned differences were maintained through successive generations of culture, the types were evidently either different species or different strains. The differences in macroscopic appearance arose from differences in length and branching of conidiophores, amount of superficial hyphal growth beyond that adjacent to the surface of the medium, and the colour of conidiophores and conidia in mass. But under the microscope each type showed similar variations in shape, size and colour of the conidia, and the types could not be distinguished from each other with certainty. Text-fig. 1, III and IV were drawn from the wheat and broccoli types respectively after 7 days' growth on beerwort gelatine at 14° C. to 16° C.; it will be seen that the microscopic characters are not distinctive, although as previously shown these types are so different in macroscopic appearance. Macroscopic differences alone being inacceptable as a basis for distinction of species, the types in question must be considered strains of *C. herbarum*, and not different species of Cladosporium. Since these strains (as they may now be termed) retained their distinctive characters on media containing different nutrient material, and of different hydrogen-ion concentrations, it seems permissible to conclude that corresponding differences of nutrients and hydrogen-ion concentrations in the juices or moisture of natural substrata will not modify the characters of a strain in nature. It follows that the different types found in nature, if under identical conditions, would represent different strains of *C. herbarum*, not variations of a single strain. That a certain strain has been found to occur more frequently on a certain type of host plant, has given rise to the conception of "prevalent strains," an idea worthy of further investigation than has yet been possible. It may serve a useful purpose if the incorrect specific names are abolished in favour of "strains of *C. herbarum*."

So far, this investigation established the fact that no different species of Cladosporium had been found on our common cereals and brassicas,



Text-fig. 1. *C. herbarum.*

I. From olive-green mass on wheat leaf.

II. Same fungus recovered from seedling after inoculation of seed; (*a*) normal form, (*b*) bud-spore form, from same shoot under same conditions.

III. Same fungus from beerwort gelatine culture 7 days old.

IV. *C. herbarum*, broccoli strain, from beerwort gelatine culture 7 days old.

(III and IV illustrate similarity of totally distinct strains.)

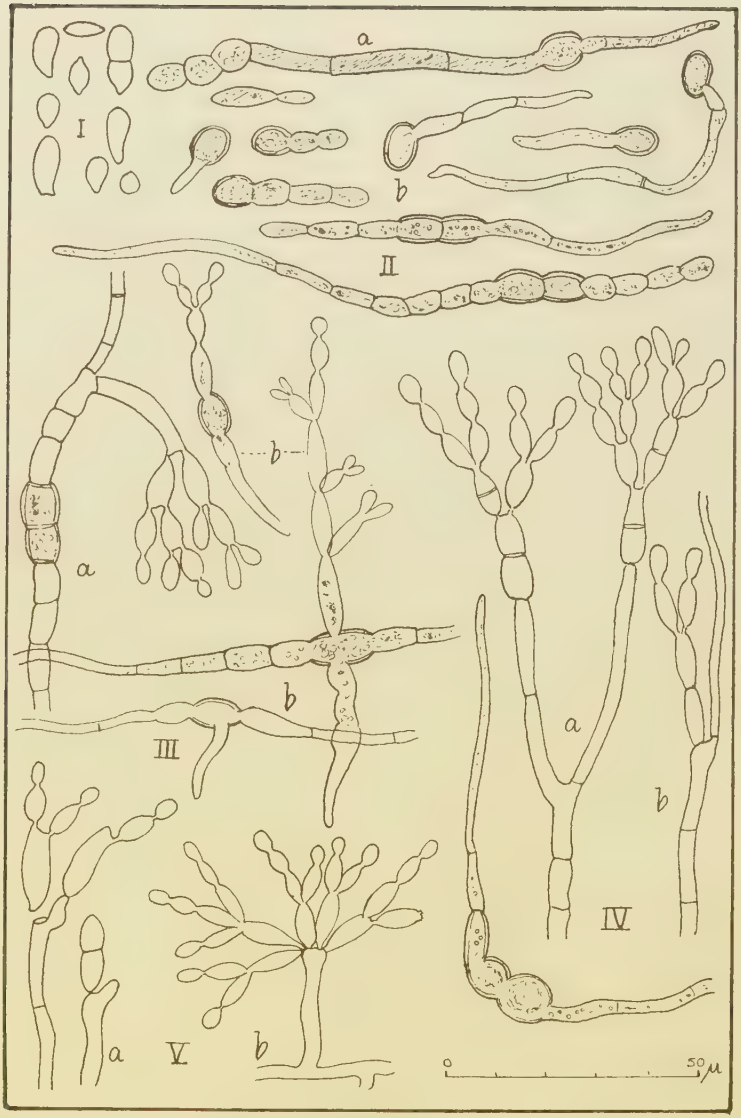
whilst three distinct strains of *C. herbarum* occurred commonly on the cereals and two others commonly on the brassicas. These strains were the only ones observed from the cultural examination of a large number of specimens, and, though they are probably not the only strains, they may be considered truly typical of the species *C. herbarum*. They therefore afforded suitable material for the next step in the investigation, viz. the study of the parasitism of the strains of *C. herbarum*.

The "Hormodendron" Stage.

The genus Hormodendron, according to systematic works, contains three species affecting cereals (see p. 194). It has long been known that Hormodendron was another form of Cladosporium, but there were differences of opinion as to the relative nature of the two forms. Schostakowitsch (11) claimed to distinguish between them by differences in heliotropic reaction, conidial walls, and type of growth in certain concentrations of potassium nitrate. Brooks and Hansford (2), however, could not so distinguish between them, and showed that they did not differ morphologically or physiologically. It was possible, however, that a biological difference existed between the two forms, for, whilst the earlier investigators, *e.g.* Janczewski (5), regarded Hormodendron as a saprophytic polymorphic form, Bancroft (1) described it as the parasitic stage of *C. herbarum*; and Massee (10) says: "The Hormodendron conidia infect living plants, and continue to produce the Hormodendron form throughout the summer season," whilst the Cladosporium form, according to Bancroft, is afterwards produced on the dead tissues. These divergent views as to the parasitic or saprophytic nature of the Hormodendron stage could be reconciled only if there existed races or forms which differed from each other biologically. The investigation of the present problem, therefore, rendered it essential to attempt the production of the Hormodendron stages of the strains of *C. herbarum* isolated, in order to study their parasitic capacity, and to ascertain whether they differed biologically or not.

Production and Parasitism of "Hormodendron."

Using the five strains of *C. herbarum* isolated, the Hormodendron form was produced from each by the methods of earlier investigators and in other ways. It was found that the profuseness of its production was correlated with abundant moisture and a suitable temperature; in cultures it abounded on very moist media, and in the open field reached its greatest profusion in moist, sheltered positions, or in damp, warm



Text-fig. 2. *C. herbarum.*

I. Spores from wheat strain as used for germination, and comparison of influence of different temperatures and nutrients on the production of "Hormodendron."

II. Germination in 2 per cent. dextrose solution, at 16° C.; (*a*) conidiophore, (*b*) conidia.

III. Germination in (*a*) tap water, (*b*) distilled water, at 16° C. to 20° C.

IV. Germination in 2 per cent. saccharose solution, at 10° C. (*a*) from a conidium, (*b*) from a conidiophore.

V. Normal form (*a*), and bud-spore form (*b*), borne together on dead cabbage tissue at 16° C.

weather, whilst it always appeared when material naturally infected by *C. herbarum* was incubated in moist chambers. The different nutrient constituents of artificial media showed no appreciable influence on its production from normal Cladosporium, even tap water and distilled water proving sufficient (Text-fig. 2, III). As stated by Brooks and Hansford(2) for the strains which they examined, the Hormodendron form consisted merely of a budding of the first-formed Cladosporium conidia. All attempts to produce the form free from the Cladosporium type of spore by culture on artificial media were fruitless, the subsequent growth always yielding some of the latter type, and not differing from the cultures derived from the normal Cladosporium on similar media (see Text-fig. 1, III, IV).

In view of Bancroft's statement that Hormodendron was produced on the living plant, special attention was devoted to attempting its production on naturally infected leaves of cereals in the field, and on artificially inoculated leaves of brassicas under a variety of controlled, favourable conditions. On these living tissues there was not produced or maintained any more definite form of Hormodendron than was the case on artificial media. The following experiment is worthy of mention.

Sterile plugs of raw potato, carrot and parsnip were used in sterile, moist test-tube chambers. In carrot and parsnip plugs only the cells actually wounded are dead, whilst under the conditions stated the potato forms on the cut surface a protective layer which is very thin and imperfect in continuity; on all these substrata, therefore, the fungus would have immediate and easy access to actually living tissues. Such plugs were inoculated from vigorous bud-sporing (Hormodendron) growths of the wheat and broccoli strains, and kept at 23° C. for 10 days, and afterwards at 10° C. to 17° C. On the carrot and parsnip there was no definite growth of the fungus at all, merely a slight browning of the tissues at the points of inoculation. On the potato there was, about the 14th day, a delicate, white mycelium, and normal Cladosporium was present as shown by the few spores then existing; this condition persisted for many weeks (see below).

Failing to obtain and maintain a definite Hormodendron form the following experiments concerning pathogenicity were performed with the nearest possible form, viz. normal *C. herbarum* when in vigorous bud-sporing condition. Brassica plants were expressly chosen for experiment in order to correspond with some former investigations upon which statements concerning the parasitism of this fungus had been founded.

(1) Reference may first be made to the results obtained on the living tissues of potato, carrot and parsnip mentioned above. After the first 10 days these inoculated plugs were kept at room temperature for 3 months. On the carrot and parsnip there was still no growth. The scanty growth on the potato plugs was found to be confined to the outer (dead) layers, the living cells below not being invaded. Cooked plugs

of these materials, on the contrary, bore abundant mycelial growth and conidia by the third day, and the plugs were eventually permeated and blackened throughout. Inoculation of plugs with normal *C. herbarum* growth, in parallel with the foregoing tests, gave exactly similar results.

(2) Cabbage and cauliflower plants were potted some weeks before use so as to be well established and healthy. Before inoculation they were cleansed and dried off quickly in a warm room; after inoculation they were covered with bell jars for the first 24 hours at a temperature of 15° C. to 18° C. Inoculum was from cabbage and broccoli strains after repeated transfers on grape-juice gelatine, in the most perfect bud-spore stage procurable. Controls, using water only, were included in each experiment.

(*a*) Inoculation by transferring conidia to drops of water placed within marked circles on the leaves, and the plants kept for 3 weeks at a temperature of 6° C. to 17° C.

(*b*) Inoculation by spraying with an aqueous suspension of conidia, and the plants grown on at a temperature of 10° C. to 28° C. In a parallel experiment on outdoor plants the latter were covered during the first 36 hours.

(*c*) Inoculation of stems at incisions made with a scalpel midway between the soil and the lowest leaf-axil, the wounds being covered for 3 days with cotton wool kept moist continuously; these plants were grown on at a temperature ranging from 10° C. to 28° C. The experiment was made in parallel on similar plants growing naturally out-of-doors.

In not one of the three experiments was inoculation followed by infection, the inoculated plants being in every respect as good as the controls. In experiment (*c*) the wounds healed with a brown layer on which traces of Cladosporium were sometimes found, but no hyphae could be found in the tissues beyond the dead cells.

These experiments showed that *C. herbarum*, even in the most vigorous bud-spore form procurable, and under conditions extremely favourable to infection, did not attack healthy living tissues. As the results were directly contrary to those obtained by Bancroft it was deemed advisable to test further the pathogenicity of all the strains in hand, but in parallel with a definite parasite so as to remove any doubt as to the suitability of experimental conditions.

Comparative Inoculation Experiments.

The parasitic fungus used for comparison was *Alternaria herculea* (E. and M.) Ell.[1], chosen after preliminary studies for the following important reasons:

[1] *A. herculea* (E. and M.) Ell. This fungus was obtained from turnip and swede leaves in the field. It causes spots with dried centres which bear conidia very sparingly; the dead centres fall out with extreme facility, and leave clear-cut holes with narrow brown margins. *C. herbarum* prevails on these margins, but the mycelium of *A. herculea* persists in the dead and the adjacent living cells. *A. herculea* yielded slightly different forms from single conidia, hence the symbols *M*1*a*, etc. in the tables and in Plate XI, fig. 4. This fungus agrees with that described by Weimer (15), and the name he uses is, with reservation, adopted here.

(1) It is an actual cause of leaf perforations in brassicas, as attributed by Bancroft to Hormodendron;

(2) *C. herbarum* occurs so abundantly at the margins of holes produced by this Alternaria that it masks the latter and appears to be itself the cause of the perforations;

(3) it may easily become included in cultures of *C. herbarum* prepared from the margins of perforations, and on the media used by Bancroft might have escaped detection owing to the extreme rarity of spore production and the similarity of the vegetative growth of the two fungi.

Experiments. Throughout the following experiments the inoculum consisted of conidia and conidiophores taken from cultures grown from single conidia of the Alternaria forms named, in some cases a mixture from such cultures being used as indicated in the tables. For Cladosporium the inoculum was taken from pure cultures of the strains named, when showing vigorous bud-spore production. The inoculum, in a little sterile water, was applied with slight friction with the smooth end of a glass rod, so avoiding wounding, and after inoculation the plants were covered with bell jars for such time as was necessary to maintain a film of moisture on the inoculated spots.

I. *On Seedling Brassicas.*

(*a*) Cabbage seedlings in the four-leaf stage were inoculated at one point on each side of the midrib of all leaves used. In pot 3 every leaf was pricked with a sterile needle on the left side of the midrib before inoculation; of each pair of plants one was inoculated on the upper and the other on the lower surface.

Pot	Fungus	"Strain"	No. of plants	No. of inoculations	No. of infections
1	*C. herbarum*	Broccoli	1	8	0
	A. herculea	1 *a*	2	16	14
	,,	2 *a*	2	16	16
2	*C. herbarum*	Cabbage	1	8	0
	A. herculea	1 *b*	2	16	12
	,,	2 *b*	2	16	13
3	*C. herbarum*	Broccoli + cabbage	2	16	0
	A. herculea	1 *a* + 1 *b*	2	16	15
	,,	2 *a* + 2 *b*	2	16	14

In not a single instance did *C. herbarum* cause infection, whilst *A. herculea* gave from 12 to 16 infections in every set. The results in pot 1 are illustrated in Plate XI, fig. 4.

(*b*) Swede and turnip seedlings were treated as were the cabbage seedlings. Of 12 inoculations with *C. herbarum* not one was followed by infection, whilst 72 inoculations with *A. herculea* gave 62 infection spots.

In both the above experiments *C. herbarum* became established at some of the needle wounds, but in none did it make any progress into the adjacent tissue so long as the leaf was healthy. The Alternaria attacked the leaves by way of the

upper and lower surfaces equally well, and made no greater progress at the wounded spots than elsewhere—in fact, several of the "misses" occurred at the punctured inoculations.

II. *On Older Brassicas.*

Brussels sprout plants were transplanted to pots and grown on outdoors until well established, with at least three good, perfect leaves between the youngest and the oldest; these are the three leaves referred to in the following tables.

(*a*) Each leaf was inoculated at four points, and the plants then covered for 4 days with bell jars, which were removed daily for aeration and atomising with sterile water when necessary to keep the inoculated spots moist; the fungi used were as follows:

Inoculum	Plant 1	Plant 2
A. herculea 1 *a* + 1 *b*	Oldest leaf	Youngest leaf
„ 2 *a* + 2 *b*	Second „	Oldest „
C. herbarum Broccoli + cabbage	Youngest „	Second „

Controls—other leaves of the same plants.

The results obtained in the two pots and their duplicates were very similar to those of Plant 1, illustrated in Plate XI, fig. 5. Infection by *A. herculea* and the complete absence of infection by *C. herbarum* on the same plants are obvious.

(*b*) In this experiment *C. herbarum* was tested against *A. herculea* on the same leaf, by applying the former on the left and the latter on the right of the midrib. The inoculated plants were covered for 2 days only, to avoid lowering their vitality unduly, and for some days afterwards were sprayed with sterile water from an atomiser. All five strains of *C. herbarum* were tested separately on different leaves, the arrangement being as follows:

Plant 1	*C. herbarum* left of midrib	*A. herculea* right of midrib
Oldest leaf used	Broccoli	1 *a*
in	Cabbage	1 *b*
Succession	Wheat	3 *a*
to	Barley	2 *a*
Youngest leaf used	Oat	2 *b*

Plant 2 as plant 1, but order of leaves reversed.

Not one of the strains of *C. herbarum* caused infection except on one leaf which was in a state of low vitality, as shown by its turning yellow a few days after being covered. *A. herculea*, except once by 3 *a*, caused infection at all points of application.

These experiments on seedling and older brassicas prove beyond doubt that under conditions which permit regular infection by a true parasite, not one of the so-called Hormodendron forms of the typical strains of *C. herbarum* exhibits any true parasitic capacity, and that this fungus does not cause spots or perforations on the leaves of brassica plants.

Discussion of the "Hormodendron" Theory.

The bud-spore stage of *C. herbarum*, described by earlier writers as Hormodendron, can be produced under a variety of controlled conditions, but it cannot be developed as an entity free from Cladosporium, either on dead or living matter. The bud-spore forms of the typical strains of *C. herbarum* here studied are non-pathogenic, though they may be termed "semi-parasitic," since they can establish themselves on unhealthy or dying tissues. The normal forms of *C. herbarum* can also do this, and, therefore, there is no difference between the normal and the bud-spore forms in their capacity for parasitism; neither is there any biological difference between the bud-spore forms of the different strains of *C. herbarum*. When established on suitable tissues the bud-spore forms do not, as stated by Bancroft, cause perforations. In experiments not recorded here, *C. herbarum*, isolated from the margins of holes in wheat, cherry and plum leaves, failed completely to cause holes when applied to healthy leaves of similar host plants; the fungus had been existing merely on the very restricted dead and dying marginal areas of holes produced by some other insect or fungal organism. It is suggested that where application of *C. herbarum* to healthy leaves resulted in infection and perforation(1), the inoculum contained some masked parasitic organism such as the Alternaria used in the experiments previously described. Further, as *C. herbarum* so frequently masks a true parasite at leaf perforations, experiments in which diseased leaf material is used as inoculum are useless as a basis for statements concerning the parasitism of this fungus. The present investigation shows that the theory of a "parasitic Hormodendron stage of *C. herbarum*" is untenable, and affords definite evidence in support of the views of those earlier investigators (p. 192) who held that *C. herbarum* is not a true parasite in any stage.

Since the Hormodendron form does not differ from the normal *C. herbarum* form morphologically or physiologically (p. 199), or as here shown biologically, there is no justification for the continued use of a generic name for this bud-spore form of *C. herbarum*. Further, as so many specimens of *C. herbarum* and its bud-spore form were examined critically, and all the bud-spore forms found yielded one or other of the strains of *C. herbarum* mentioned, it would appear that there are no such distinct organisms as *H. cladosporioides*, *H. hordei* and *H. herbarum*; therefore, all the bud-spore forms occurring on the cereals in this country should be relegated to the genus Cladosporium, and probably

to strains of the species *C. herbarum*. Application of this principle of dispensing with the generic name Hormodendron is seen in the naming by Dowson (4) of the new species *C. album*.

C. herbarum in relation to "thinning out" and "deaf ears" in wheat.

The fact that *C. herbarum* was not parasitic in its normal or its bud-spore (Hormodendron) form on brassicas, still left the original wheat problem unsolved, though it indicated that the troubles were probably not due to this fungus. The following experiments were carried out in order to ascertain the effect of *C. herbarum* on wheat under various conditions. The three strains isolated from the cereals were tested together, the inoculum being a mixture of conidia taken from cultures bearing the greatest proportion of the bud-spore form obtainable.

Contaminated Grain grown under Various Conditions.

The germination capacity of the wheat used, after external disinfection of the best grains, was 99 per cent. In all the experiments inoculation of the disinfected grains was done by one of two methods: (1) contact of the still moist grains with cultures of the fungi; (2) immersion of the grains in an aqueous suspension of conidia. Control and inoculated grains were then placed on dry, sterile sand, and kept in moist chambers for one week to give opportunity for the fungus to become well established on the outer covering of the grains; these were then used as follows:

(1) *Growth under Abnormal Conditions.* Lots of 25 grains from each of the control, contact inoculation, and immersion inoculation sets, all in duplicate, were grown on sand in porous dishes; for the first week they were held in dark, moist chambers, and for the following 3 weeks exposed to light in a moist atmosphere under glass bell jars. The experiment was repeated one month later. At the end of one month the portions of stems between the first leaf-axil and a point about half-an-inch above the sand level were cut out, disinfected externally by a proved method, and incubated, each in a sterile, moist test-tube. The results obtained were as follows:

	Control	Contact inoculation	Immersion inoculation
Germinated grains	100	100	98
Shoots yielding *C. herbarum*	0	88	83

(2) *Growth in Wet Soil.* In each 12-inch pot, containing steam-sterilised soil, 12 grains from each of the prepared groups were planted, and grown on in an unheated greenhouse from April to June; the plants were kept moist at the roots continuously. All the grains yielded plants, but those from inoculated grains were less well-grown than the controls, the lower leaves dying off sooner, and more numerous tillers were produced. The plants were reduced in number by taking the aerial part of two plants from each pot, at intervals of 2 weeks, to the laboratory for examination. No trace of the fungus was found in any of the parts above soil level,

but it was obtained from the underground parts of each of the four plants and their duplicates left growing until the end of the period.

(3) *Growth under Normal Conditions.* Using 14-inch pots and sterile soil, the grain was planted, germinated and brairded with the soil slightly moist. The three pots, in duplicate, were then sunk in the soil in a "cage" plot outdoors, eight seedlings being left in each pot to grow on to maturity; water was given only during a period of drought. Throughout the season all the plants were poorer than those in the field, but those from the inoculated grains were no worse than the controls. At maturity the following results were obtained:

Plants	Spikelets per ear. Av. No.	Grains per spikelet. Av. No.	Weight of 1000 grains in gm.
Control	13·9	2·44	28·7
Contact inoculation	14·4	2·5	31·8
Immersion inoculation	14·3	2·42	29·75

The first of these experiments showed that under abnormal conditions of growth, resulting in weak, semi-etiolated shoots, 85 per cent. of the seedlings contained the fungus in the still living shoots at the end of one month. With the aid of a lens its presence could be detected by faint, brownish streaks in the outermost sheaths, whilst sections stained and mounted for examination under the microscope showed it to occur in the parenchyma only, and this of the outer layers, not centrally; thin sections cut from the same shoots after external disinfection, placed on plates of media, gave *C. herbarum* consistently, thus verifying the nature of the fungus revealed in the tissues by staining. Evidently the fungus could exist in unhealthy, living tissues. In the "wet soil" experiments the conditions resulted in unhealthy growth of the underground parts, for the fungus extended from the grains to the primary and secondary roots, and developed considerably about the buried base of the stem. As shown in the experiment (2), the presence of the fungus undoubtedly exerted an adverse influence upon the growth of the plants, but not to such extent as to stop their development completely. As shown in experiment (3), however, grain similarly infected and grown in well-drained soil yielded healthy plants, without reduction in ears or number and weight of grains. Therefore, given properly matured seed-corn, the presence of the fungus thereon proves harmful only under such bad soil conditions as are themselves the primary cause of unhealthy root growth, leaving the latter susceptible to fungal attack.

Inoculation of Aerial Parts.

(1) *Effect on Seedlings.* Wheat, barley and oats were grown, six per pot, to a height of 8 in. The plants in one pot of each cereal were inoculated by spraying with a suspension of conidia mixed from the different strains, and in a second pot

by inserting a drop of the inoculum between certain leaf-sheaths and the internodes; the plants in the third pot, treated in both these ways with water, served as controls. The plants were covered for 3 days after inoculation, with daily aeration, then grown on in an unheated greenhouse until they showed signs of ears. These young plants, which at the time of treatment bore sound leaves only, remained totally free from infection.

A similar experiment with plants 12 in. high when treated, showed the fungus to be established on some of the lower leaves, which were probably adversely affected by being covered, and these leaves died back rather earlier than corresponding leaves of the controls; but these plants suffered no appreciable harm, for at earing time they appeared equal to the controls in every respect. It appeared that the fungus merely hastened slightly the death of leaves which were more slowly losing vitality naturally.

(2) *Inoculated Plants grown to Maturity.* Wheat, 18 in. high, was inoculated as in the preceding experiment, but these plants were covered for 4 days, with daily aeration, and thereafter grown on outdoors, the three pots and the duplicates being sunk into the soil of a "cage" plot. Eight plants were allowed in each pot, and when mature, the eight main ear-bearing stems from each pot were examined, with the following results:

	Control	Inoculated by spraying	Inoculated in leaf-sheaths
Av. No. spikelets per ear	19·2	20·0	19·8
„ of grains per ear	52·1	49·3*	53·2

* Corn thrips did noticeable damage.

The growth of the inoculated plants throughout the season was quite as good as that of the controls, and the final results recorded above show that *C. herbarum* applied externally to growing wheat plants had no deleterious effects.

(3) *Inoculation of Ears.* Wheat was grown in the open in a "cage" plot, in three beds separated by wide paths, along which screens of double muslin were erected before inoculation to minimise contamination by wind-blown spores. Bed 1, on the windward side, was the control; in bed 2, ears were inoculated after flowering when ovaries were swelling; in bed 3, ears were inoculated during flowering when stamens were protruding abundantly. In each of beds 2 and 3 fifty ears were inoculated by spraying with a suspension of conidia, ears in bed 1 being sprayed with water. Further, in each of beds 2 and 3, the lowest and uppermost spikelets were cut from ten ears, and the remaining spikelets inoculated by allowing a drop of inoculum to fall from a dropper between the glumes opened with forceps, when the grains within were definitely enlarged. All treated ears were enclosed in thin, grease-proof paper bags, closed around the "neck," and removed after 3 days, the ears being then left to develop under ordinary conditions.

Observations at weekly intervals during subsequent growth revealed somewhat more Cladosporium on the spray-inoculated ears than on the water-sprayed ears, but the fungus occurred mainly on the chaffy scales towards the centre of the spikelets, and which in no ears throughout the beds ever bore grains within. The grain was harvested before complete maturity, in order to avoid possible casual infection at the end of the season. The ears inoculated by spraying gave grains equal in average number and weight to those of the water-sprayed ears, whilst those inoculated by drops gave 3 per cent. fewer grains than a corresponding number of

similar spikelets on water-sprayed ears (no control was carried out for the drop inoculation)—a reduction of no significance.

Observations on the grains themselves did not show any visible development of Cladosporium upon them more than was to be found on any grain from the plot; such of the fungus as was present occurred as bits of mycelium and occasional conidia amongst the hairs at the apices of the grains. The disease known in America as "Black Point" did not appear in these ears inoculated with Cladosporium, indicating that in this country, as in America, *C. herbarum* is not the cause of such disease under normal conditions.

The experiments described proved that at no stage of its growth is healthy wheat attacked in its aerial parts by *C. herbarum*. When applied freely the fungus did not check the growth of the plants, the production of ear-bearing stems, or the production of grain in the ears. When applied to the ears it did not cause sterility of individual florets, or the production of shrivelled, diseased grains, whilst the grains themselves showed no greater amount of the fungus than was normal for the district and season.

C. herbarum *in relation to the Wheat Crop.*

The only definite strains of *C. herbarum* yet isolated from cereals have been tested for pathogenicity to wheat, under such conditions as to favour any parasitic capacity of the organism. The strains were not tested separately, because they had shown themselves equally lacking in parasitic capacity in critical trials previously. The fungus established itself readily on the moist outer coats of ripe grain, but did not do so on young, growing grains while in the ears. In dry, harvested grain the fungus is found as mycelial segments and sometimes conidia amongst the apical hairs, and as microsclerotia partially embedded in the pericarp; this occurrence appears to be due to weather conditions during the later period of ripening, which favour the growth of the fungus whilst delaying the final stages of maturation of the grains. Except when previously injured by other parasites (*e.g.* Fusarium, or insects), or the grain has ripened under very adverse conditions, the embryo itself is not affected by the fungus, hence even badly affected grains show a satisfactory germination capacity. The use of grain badly affected with *C. herbarum* for sowing, however, is inadvisable, because the presence of the fungus shows that the grain was ripened under poor conditions, and badly ripened grain yields weakly seedlings; such weak, poor seedlings are susceptible to attack by even such a "weak parasite" as this fungus. The poor seedlings produced by the ill-matured grain have then to contend with the further disadvantage of the semi-parasite. Grain

which is well developed and sound except for the presence of *C. herbarum* may safely be used for sowing under normally good conditions of soil and climate, but if the soil and climatic conditions are such as to cause the seedlings to be weak or unhealthy, the presence of the fungus is a disadvantage, since it will attack such seedlings and further adversely affect their growth. The decision whether or not to use affected grain for sowing must, therefore, depend upon the general condition of the grain, and the soil and probable climatic conditions under which its early growth would be made. If the primary causes of disease, viz. poor "seed" and bad growing conditions could be eliminated, *C. herbarum* could be ignored. It has been found in preliminary work by the writer, that the usual "pickling" processes are beneficial to good grain on which *C. herbarum* is established externally. These processes have been found to kill the adhering mycelium and conidia, and greatly to retard and reduce the growth from the microsclerotia. It is considered that the processes facilitate the establishment of healthy seedlings, not only by preventing any early attack by the fungus, but by reduction of the vitality of the fungus which does develop; treatment with copper salts has given the best results.

C. herbarum is generally plentiful during a wet season on the aerial parts of wheat plants. It becomes established on dying tissues, such as aged leaves, and scorched tips of leaves and glumes, and probably extends into the adjacent parts as these lose vitality, so hastening, to some extent, their final collapse. The statement by Mackie(8), that Sooty Mold (*Hormodendron cladosporioides*) causes serious losses in wheat fields in the coastal districts subject to spring and summer fogs in California, supports this view; the conditions are such as would favour "scorching" of the tips of leaves and chaff, and, by the moist atmosphere, tend to lower the vitality of all parts of wheat plants. Under normal conditions in our country, the damage so done appears to be slight, for in experimental trials it was not appreciable; on the other hand, when wet weather prolongs the stage of "ripening off," the fungus prevails on the ears and causes the grains to be poorer than they would have been in its absence. Similarly, whole plants which are unhealthy from some other cause—insect or fungus—may be heavily infected with *C. herbarum*. Its presence on plants which "ripen prematurely," and on others which yield few and shrivelled grains, is a clear indication of an unhealthy condition induced by some more obscure cause. "Premature ripening" or "whiteheads," sterility of individual florets, and "deaf ears" are not caused by *C. herbarum* either occurring on the grain

sown or reaching the aerial parts of the plants by casual spore distribution.

Summary.

The only types of Cladosporium found occurring on our four cereals were strains of *C. herbarum*, not different species.

Five strains of *C. herbarum*, tentatively termed "prevalent strains" of wheat, barley, oats, cabbage and broccoli, were isolated from these plants respectively.

The bud-spore forms, or so-called Hormodendron stages, of these strains do not differ in parasitic capacity from the normal Cladosporium forms; they do not cause perforation of leaves.

None of these strains of *C. herbarum*, and they may be regarded as typical of the species, is parasitic in any stage or form on brassicas or cereals; they are all "semi-parasitic," establishing themselves on, and thereafter hastening the death of, dying tissues.

C. herbarum is not the cause of "thinning out," "premature ripening (whiteheads)," or "deaf ears" in wheat.

The writer's thanks are tendered to Mr F. T. Brooks, M.A., for assistance in the preparation of this paper for publication.

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EXPLANATION OF PLATES X AND XI.

PLATE X.

Comparison of strains of *C. herbarum*, from broccoli (Br), barley (B), cabbage (C), wheat (W), oat (O).

Fig. 1. Five strains on malt gelatine.

Fig. 2. Five strains on wheatmeal agar.

Fig. 3. Two strains on synthetic agar with dextrose, showing the retention of the macroscopic characters at *p*H values 5·5, 6·6 and 7·8. These strains are not distinguishable microscopically as shown in Text-fig. 1.

PLATE XI.

Illustrating comparative inoculation experiments.

Fig. 4. *C. herbarum* (*Cl*) non-parasitic, *A. herculea* (*M* 1, *M* 2) parasitic, under the same conditions on cabbage seedlings.

Fig. 5. *C. herbarum* (white tag) non-parasitic, *A. herculea* (pink, left, tag = *M* 1, black tag = *M* 2) parasitic, on leaves of older brassicas.

(*Received October 8th*, 1927.)

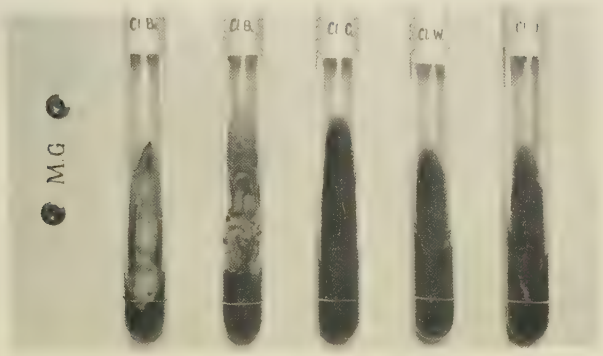

Fig. 1.

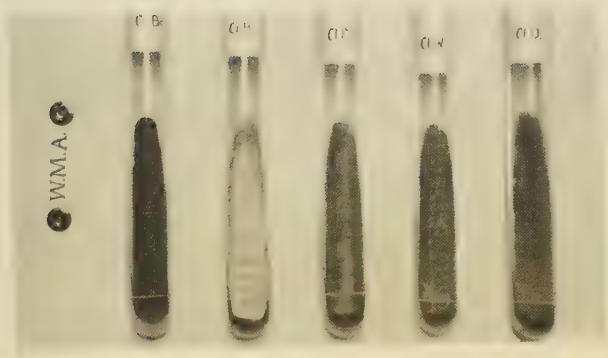

Fig. 2.

Fig. 3.

BENNETT.—On *Cladosporium herbarum* (pp. 191–212).

Fig. 4.

Fig. 5.

BENNETT.—On *Cladosporium herbarum* (pp. 191–212).

ON TWO SPECIES OF FUSARIUM, *F. CULMORUM* (W. G. SM.) SACC. AND *F. AVENACEUM* (FRIES.) SACC., AS PARASITES OF CEREALS

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(With Plates XII and XIII, and 2 Text-figures.)

CONTENTS.

Introduction.

In a previous publication(6) it has been shown that the disease known in the north of England as "thinning out" and "deaf ears" in wheat crops is not due to *Cladosporium herbarum*, as suggested by some earlier writers. The present paper deals with the true causal organisms. The investigation of the disease started in the autumn of 1923, in connection with a farm where the trouble had been persistent and had caused much loss during the preceding 10 years. Subsequent observations showed that the disease occurred to a greater or less extent on many Yorkshire farms; and during 1926 and 1927 in Durham, Northumberland and Cumberland numerous cases occurred where the loss of crop was 50 to 75 per cent. During these two seasons, in which the rainfall was greater than the average, losses of 10 to 20 per cent. of the wheat were extremely

common in the northern counties. The economic importance of the disease, therefore, is considerable.

Having disposed of Cladosporium as a possible causal organism of the disease, there remained, according to the available information bearing on such trouble in this country, only Ophiobolus as an acknowledged cause. Extensive observations in the field showed that whilst some diseased plants were attacked by Ophiobolus, others were quite free from this and from "allied"[1] fungi, and showed only some species of Fusarium at their bases. Towards the end of the season 1924, when the presence of the various fungi could be observed with some degree of certainty, counts were made on four farms situated some miles apart, to compare the relative frequency of Ophiobolus and Fusarium. As shown in the following table Fusarium appeared alone in approximately 40, 45, 10 and 25 per cent. of the diseased plants at the respective centres:

Farm	Loss of crop %	Specimens examined	*Ophiobolus* alone	*Fusarium* alone	*Ophiobolus*, *Fusarium*, etc.
Bardsey	20	72	20	28	24
Follifoot	10	40	4	18	18
Crimple	80	60	39	7	14
Garforth	5	100	25	27	48

From each of these four centres "deaf-eared" plants were removed bodily to pots of sterilised soil, and were kept until winter, some in an unheated greenhouse and others in a garden. Of these, the plants which showed no signs of Ophiobolus at the bases when taken from the fields did not develop this fungus subsequently, but gave Fusarium only, thus indicating the probable correctness of the counts tabulated. In several more recent cases observed by the writer Fusarium alone occurred, Ophiobolus and "allied" fungi being totally absent throughout the crops. These facts indicated the probability that Fusarium was a primary causal organism of the disease in question, but whilst this fungus has been recognised abroad (p. 238) as a cause of "foot-rot" no record could be found referring to any connection between the "foot-rot" and the "deaf-ear" trouble. The only information available bearing upon English conditions, apart from that given by W. G. Smith(18), was a note[2]: "A Fusarium foot-rot was common in 1920,

[1] For the purpose of this investigation plants showing the following fungi, whatever their pathogenic nature may be, were included with the "allied" group of column 6: Leptosphaeria, Pleospora, Alternaria, Helminthosporium, Cephalosporium, Phoma.

[2] A. D. Cotton, Path. Lab., Harpenden, in correspondence 10. iii. 22.

but its relationship to the host and other species was not investigated." Under these circumstances a detailed investigation appeared desirable.

Isolation and Pathogenicity of two Species of Fusarium.

Wheat plants showing the symptoms of "thinning out" and "deaf ears," as described on p. 240, but entirely free so far as could be observed from Ophiobolus and "allied" fungi, were selected from various centres. From the fractured bases of the stems, from the remaining "foot" and roots, and from the first, and sometimes the higher internodes, Fusarium was isolated with regularity. Such portions, after external disinfection and incubation, yielded a white mycelium, sometimes having a pinkish tinge, which bore abundant conidia, and occasionally small, bead-like masses composed of adhering conidia. From the conidial masses two different species were obtained without difficulty, but the two species could not be distinguished by means of their commoner conidia of the aerial mycelium until after considerable time when cultures from them reached a stage similar to that of the correlated conidial masses. Single-spore colonies[1] were obtained by transferring conidia from one drop of sterile water to another successively, under aseptic conditions so far as practicable, using finally hanging drops which, on microscopical examination, were found to contain a few clearly isolated spores only. Such drops were utilised for dilution in a malt gelatine medium. From the edges of each of six colonies (when there were so many) on a plate, transfers were made to four different kinds of media, and exact agreement throughout the subsequent growth was taken as a criterion of satisfactory isolation.

All material affected as described above, which yielded Fusarium, gave one or both of two species only of this fungus, *F. culmorum* (W. G. Sm.) Sacc., and *F. avenaceum* (Fries.) Sacc.[2]. Whilst not suggesting that these are the only species which can cause disease in our wheat crops, they are undoubtedly the predominant pathogenic species in the north of England. Since a number of species of Fusarium are considered to be soil saprophytes, the two species named were tested at the same time and under the same conditions to ascertain whether one or both were parasitic, and if both, to compare their virulence, respective effects, and host range so far as concerned the four common cereals, wheat, oats, barley and rye, as described in the following records.

[1] The term "colony" is used for the growth on a plate arising from a single spore.

[2] See pp. 225–232; before identification and verification of the species they were labelled *F* 1 and *F* 2, and these labels signify the respective species in the illustrations.

Infection of Seedlings from Seed or Soil.

For these experiments the cereal grains were disinfected externally[1], and the inoculation following was by two methods, each leaving conidia and fragments of mycelium adhering to the grains; (1) contact of the still moist grains with cultures of the fungi, and (2) immersion in an aqueous suspension of conidia.

Under Abnormal Conditions.

The grains were planted on sterile sand in porous dishes, held in dark, moist chambers for 5 days, next watered and grown there for 5 days, then for 3 weeks exposed to daylight under glass bell-jars. At the end of the period the portions of stem between the first leaf-axil and a point about half-an-inch above sand level were cut out, disinfected externally, and incubated each in a moist test-tube. The results of four experiments, dealing with 25 grains per dish each time, were as follows:

Dishes		Seeds germinated	Loss of germination	Seedlings badly diseased	Shoots incubated	Shoots yielding the fungus	% of growing seedlings attacked
			Wheat—Yeoman II.				
Control	(*)	97	—	—	—	—	—
F. culmorum	(1)	76	21	20	66	58	88
,,	(2)	88	9	12	76	72	98
F. avenaceum	(1)	96	—	12	94	54	58
,,	(2)	96	—	4	96	36	35
			Barley—Plumage.				
Control	(*)	100	—	—	—	—	—
F. culmorum	(1)	76	24	18	76	74	97
,,	(2)	95	5	11	95	88	92
F. avenaceum	(1)	94	6	20	91	58	65
,,	(2)	96	4	11	92	38	41
			Oats—Svälof Crown.				
Control	(*)	100	—	—	—	—	—
F. culmorum	(1)	96	4	—	96	96	100
,,	(2)	100	—	2	98	98	100
F. avenaceum	(1)	100	—	1	98	48	50
,,	(2)	100	—	—	100	21	21
			Rye—Essex Giant.				
Control	(*)	92	—	—	—	—	—
F. culmorum	(1)	71	21	13	60	46	96
,,	(2)	70	22	8	62	53	86
F. avenaceum	(1)	70	22	2	68	54	80
,,	(2)	83	9	—	84	66	79

(*) From each control 40 (*i.e.* 10 per dish) shoots were taken at random for incubation not one yielded Fusarium.

(1) = contact inoculation, (2) = immersion inoculation.

[1] By soaking overnight in water at room temperature, then treating for 3 minutes with 0·2 per cent. solution of mercuric chloride in 50 per cent. alcohol, and finally thoroughly washing with sterile water.

The results show that both *F. culmorum* and *F. avenaceum*, established on otherwise sound seed corn reduce the germination capacity severely in rye, and to some extent in barley, but *F. culmorum* alone did so for wheat and oats. In all the cereals, however, between 86 and 100 per cent. of the shoots were attacked by *F. culmorum*, and 35 (the average) in oats and 80 per cent. in rye by *F. avenaceum*. Very many seedlings were obviously severely affected or actually dead, whilst in those less badly affected the fungus was found in the tissues well above sand level. The germination capacity of a commercial sample of seed, more particularly wheat and rye, therefore, may be low merely on account of the presence of these fungi externally, and the fungi may pass from such seeds into the seedlings which do grow.

Under Normal Conditions.

Ten (rye 9) seedlings were raised per pot (7-inch), in duplicate, in loamy soil sterilised by autoclaving. Inoculated grains were planted in sterile soil, and externally disinfected grains were planted in inoculated soil. For the controls sterile, cooked wheat grains were added to the soil, and for soil inoculation cooked wheat grains on which the respective fungi were growing were added. The plants were grown in an unheated greenhouse from February to June.

Wheat. The control plants at 4½ months were 18 in. high and thickening for earing. *F. culmorum* caused a loss of 10 per cent. of seedlings before one month old, and the remaining plants were very backward, until at 4 months they began to die back. *F. avenaceum* caused less early loss of seedlings, but by the end of the second month 50 per cent. had died off. In another lot of pots, where the soil was kept moist constantly, the damage was extremely severe, the early losses alone being from 20 to 50 per cent. of seedlings. The respective species of the fungus were recovered, after each method of inoculation, from the base of every seedling, and from a part of the stem well above soil level of those seedlings which died off in early growth.

Barley. The control plants grew well, at 3 months being 15 in. or more high, and showing the tips of the beards. *F. culmorum* prevented germination of 20 per cent. of the seeds, and affected every seedling, checking the growth, and causing the plants to bleach and die off before reaching a height of 12 in., or showing signs of beards. *F. avenaceum* prevented germination of 25 per cent. of the seeds, and affected every seedling which grew in similar manner to the former species. For both species soil inoculation resulted in greater injury to the young seedlings, and earlier death of the plants, than did seed inoculation, probably because in the former case the attack on the bases was more complete. The diseased base and stunting of growth of barley seedlings is illustrated in Plate XII, fig. 2.

Oats. In the control pots all seeds grew and produced healthy plants showing their first spikelets when 3 months old. Under both seed and soil inoculation with *F. culmorum* 40 per cent. of the seeds failed to give plants which reached the earing stage, the plants then existing being stunted in size and bleaching out when the controls started to protrude panicles. *F. avenaceum* gave almost identical results,

except that under seed inoculation fewer plants died off, and the established ones had rudimentary ears in the upper sheaths at the time they bleached out.

Rye. Whilst in the control pots all seeds grew to healthy plants with ears just breaking the sheaths at 2½ months, one plant only reached this stage after soil or seed inoculation with *F. culmorum*, the others dying off when 8 or 9 in. high. *F. avenaceum* was much less virulent, for though all the plants were diseased, the majority continued growth until approaching earing stage when bleaching commenced. The bleaching appeared first in these plants in the central (last emerged) leaf, and extended rapidly throughout each main shoot. One set of these plants is shown in Plate XII, fig. 3.

Under normal conditions for plant growth both species of Fusarium, whether in the soil or on the seed, affected all four cereals. Speaking generally, *F. avenaceum* is less virulent than *F. culmorum*, doing less damage to the plants in the early stages of their development, except under moist soil conditions, but eventually the effects on the established plants are alike for both species. The experiments show that there is some loss caused by prevention of germination of seed, but there is greater loss later by the dying off of seedlings, commonly termed "seedling blight," wheat and rye suffering more severely than barley and oats. At a still later stage, the established plants of each kind of cereal show similar ill-effects. They are invaded at and below soil level, the crown[1] and many of the roots being brown and soft. When the soil is persistently moist the rotting of the underground parts is more pronounced, and the fungus may extend within the tissues an inch or more above soil level, and there emerge at the surface whilst the seedling is still living (Plate XII, fig. 4); very few seedlings survive when so affected. Diseased plants which are not killed off as seedlings make poor growth, are of a sickly yellowish green colour, and the leaves (even the youngest) wither from the tips downwards, the extent depending on the severity of the basal infection. Such plants are found in affected field crops in spring, the condition being known locally as "spring yellows" (Plate XII, fig. 1). Though some may die out at this stage, the majority struggle along in a backward condition until earing time, when the disease shows its more obvious effects.

Effect on the Maturation of Cereals.

In these experiments the cereals were grown in 12-inch pots, this giving sufficient room for development of roots of the nine plants in each, and the soil being such as to promote good growth to maturity. After brairding the pots were placed in the open, and watered through the season in such a way as to approach field conditions

[1] "Crown" meaning the somewhat conical base of the stem which bears the normal (coronal) roots, and in its early stage, the lateral branches or tillers.

as closely as possible. The tillers were thinned out on June 20, to favour grain production in the main shoots, but the diseased plants produced new tillers abundantly, these remaining green (though short) long after the main shoots were bleached as described below; this "grassy" growth is visible in the illustrations on Plate XIII. The state of the plants about flowering time (July 20th) is shown in Plate XIII, figs. 6 and 7, the barley and rye, not here illustrated, showing equally striking results. The following record was made when the grain was mature, between August 12th and 24th, for different cereals.

Wheat. The controls, 9 plants about 3 ft. high, were shooting the ears by July 1st, and eventually produced 18 ears each 4 in. or more long, with sound, well-developed grain. *F. culmorum*, applied to the seed, reduced the plants to 7, and, in the soil, to 8; in each of these pots 3 ears only emerged from the sheaths. Seed inoculation with *F. avenaceum* resulted in 8 plants, which bore 5 ears, and soil inoculation in 8 plants with 1 protruded ear, 3 partially protruded, and 4 showing tips only. For both species the tallest stems did not exceed 2 ft. in height, all became "prematurely ripe" or bleached, and many failed to extrude the ears. The few ears which did emerge were between $1\frac{1}{2}$ and 2 in. long, became bleached along with the stems, and contained no grain whatever. The bases of all the plants were in a state of dry rot, and the respective species of Fusarium were recovered from the interior of all the crowns and from some discoloured first aerial internodes (Plate XIII, fig. 6).

Barley. By July 10th the controls were showing ears, whilst the plants under inoculation, though nearly as tall, were markedly thinner in stem, yellowish in colour, and showed no sign of earing. At the end of the season (August 20th) the control plants were $2\frac{1}{2}$ ft. high, with 18 ears each more than 3 in. long, and bearing well-developed grain. *F. culmorum* and *F. avenaceum*, applied in the soil, each reduced the number of plants by 25 per cent., and their growth to between $1\frac{1}{2}$ and 2 ft., whilst the stems bleached rapidly after July 20th. No ears emerged fully from the sheaths, nor did they contain any grain whatever.

Oats. The panicles of the control plants were appearing on July 13th, and by August 20th they numbered 19, all bearing good grain, and borne on stems 3 ft. or more high. Soil inoculation with each of the two species of Fusarium reduced the number of standing plants by two, and so checked the growth that none exceeded 2 ft. in height, and all were small in leaf and of poor colour. Under *F. culmorum* there were 4 partially and 4 fully emerged panicles, whilst *F. avenaceum* reduced the ears to 3 partially protruded ones only. All these ear-bearing shoots bleached at earing time, and their spikelets were devoid of grain (Plate XIII, fig. 7).

Rye. Ears were appearing on control plants on July 7th, the 9 plants eventually reaching a height of $3\frac{1}{2}$ ft. and yielding 23 full-sized ears filled with good grain. Soil inoculation with each species of Fusarium reduced the number of plants by 3, and none reached a height of 2 ft. Under *F. culmorum* 7 small ears appeared, and under *F. avenaceum* 6 wholly or partially emerged, but none of them bore grain.

Wheat, barley, oats and rye, sown in inoculated soil, or sown as infected grain in clean soil, are attacked basally by both *F. culmorum* and *F. avenaceum*, which kill off some proportion of the plants, and cause in others a kind of dry rot of the crowns and slight browning and softening of the lower parts of the main stems, a condition known as

"foot-rot." Under normal growing conditions this attack shows its effects most clearly at earing time. The ear-bearing stems may then show partially or fully protruded ears, but instead of progressing to maturity the stems and ears become bleached or "prematurely ripe," and the ears are either devoid of grains or contain only a few, small, shrivelled ones. Thus both species of Fusarium produce the condition of "whiteheads" and "deaf ears," and not in wheat only, but also in barley[1], oats and rye. This disease is, therefore, one of the causes of that condition in oats where barren spikelets occur in the whole or a definite part of the panicle, a condition which, in the absence of evidence of insect attack, has generally been regarded as an unsolved entomological problem. Though "thinning out" did not occur in these pot experiments, the rotting of the crowns indicated that under natural conditions this phase of the disease would appear.

Infection of Aerial Parts.

(1) *Inoculation of Growing Stems.*

Wheat plants about 9 in. high were inoculated along stretches of successive leaf-sheaths, by applying a drop of an aqueous suspension of conidia from sporodochia of *F. culmorum* and *F. avenaceum* respectively. In another experiment larger plants were similarly inoculated, but a second inoculation was made after an interval of 2 weeks. After the inoculations the plants were covered for from 3 to 7 days in different trials, then left uncovered. In a third experiment diseased seedling shoots (obtained from other experiments and verified after incubation) were applied to the stems, and covered with cotton wool which was kept moist for 3 days; to the same stem sections inoculum was applied again at the end of 1 month. All the plants used were grown on when uncovered in an unheated greenhouse at a temperature of 12° C. to 18° C. for periods ranging from 10 days to 2 months. Some of the plants in every pot served as controls by treatment with sterile water.

Application of the fungus to the stems of young, healthy wheat plants made no difference to the subsequent growth, the leaves whose sheaths were inoculated, and the new leaves produced later, resembling those of the controls. The only visible stem lesions, two faint brownish areas, followed *F. culmorum* applied on dead seedlings under cotton wool—a process very different from natural infection. But when the stem sections were cut out, disinfected externally, separated into leaf-sheath and internode proper, and incubated, many of the sheaths showed that *F. culmorum* and *F. avenaceum* were established therein although

[1] A summary of this paper was read at the Conference of Advisory Mycologists, December 1926; various causes of "blindness" in barley were dealt with by other investigators.

no visible lesions indicated this. On the contrary, the true internodal portions were perfectly free from invasion. It may be concluded that both species of Fusarium become feebly established on the green stems of wheat when conditions are favourable to infection, but that unless the whole of the tissues are unhealthy from some other cause the fungi do not penetrate the central growing tissue; therefore, "whiteheads" and "deaf ears" are not a result of infections of stems during the growing stage. That the fungi readily attack more mature tissues is evident from field and experimental observations. These features are discussed further in support of a hypothesis advanced later.

(2) *Inoculation of Ears.*

Inoculation of ears was carried out at opposite ends of a field, the varieties in these headlands being mixed, but mainly Rector. The inoculum was a suspension of conidia from sporodochia, applied by means of an atomiser. Certain ears were covered with sterile test-tubes (8″ × 1″), loosely plugged with sterile, moist cotton wool around the "necks" of the plants, and supported in groups of four by means of wooden stakes. For each species of the fungus, and at each time of inoculation, 12 ears were left uncovered, 6 were covered for 3 days, and 6 for 7 days. Control ears were sprayed with sterile water and covered for similar periods. The first set of inoculations was in the late flowering stage, when anthers were protruding from the lower part of the ears (July 9th), the weather for the 9 days following being marked by much bright sunshine (average 8·1 hours) and high temperature (min. 11–14° C., max. 18·5–24·5° C.). The second inoculations were made 1 week later (July 16th), when flowering was over and many florets showed definitely enlarged ovaries; the first 2 days fell in the hot and bright period, but the succeeding 10 days were warm and extremely wet.

The results of these experiments give valuable guidance concerning the natural occurrence of Fusarium disease in the aerial parts of wheat. The control ears, sprayed with water and covered with glass, showed a slightly bleached appearance, and though their subsequent growth and grain production did not suffer, the glumes assumed a ripe or straw colour some time before those of normal ears, and there was a distinct tendency to shed these glumes and exposed grains by harvest time. The ears inoculated and covered for 3 days by that time showed infection of the majority of spikelets, whilst those covered for 7 days bore considerable mycelium also. Both *F. culmorum* and *F. avenaceum* became established, and there was no apparent difference in the virulence of the two species, or in the nature and appearance of the lesions produced. Their effects may, therefore, be described together.

The actual point of infection, whether natural or artificial, and on glume, leaf or stem, under moist conditions appeared first as a deep-

brown dot surrounded by a paler zone. The pale zone progressed gradually outwards, followed by the darker colour, leaving the original point of infection as a straw-coloured or bleached spot of gradually increasing size. Numerous infections together appeared as a light-brown or straw-coloured area with deeper-brown markings, and finally as brownish or bleached patches. These patches were strikingly clear in a moist atmosphere, whether that of the tubes or of the open air in wet weather. They varied in shade from reddish brown to nearly carmine, and bore externally a delicate, white, cottony mycelium. The ears, as a whole, showed this brown discoloration on the tips, along the edges, and between the veins of the glumes, and also at the bases of the spikelets on their outer sides and on the adjacent portions of the rachis. In natural infection these parts are usually affected separately. The symptoms of the disease under dry atmospheric conditions contrasted strongly with those shown in a damp atmosphere. When the inoculated ears, after being covered for 3 or 7 days, were exposed to dry, sunny weather, the brown discoloration became so faint that infected ears or spikelets were not easily recognised except by their bleached appearance in contrast with healthy ears. The mycelial growth disappeared, except between the pales, and occasionally at the bases of spikelets. In general, mycelium occurring on the various parts in a damp atmosphere left sporodochia on its disappearance in dry weather. The sporodochia, frequently termed "mucous mould," occurring mainly between the glumes and pales, became of gum-like consistency. These structures were of coral colour in *F. culmorum*, and apricot colour in *F. avenaceum*; but as the colour was not constant, and the other symptoms of attack were so similar, the recognition of the species present demanded microscopical examination. The ears inoculated but left uncovered resembled the covered ones described, differing only in having fewer discoloured areas. That the dry weather merely checked the progress of, but did not cure, the disease, was shown by the reappearance of the clearly marked symptoms on all the inoculated ears when the wet weather period followed. It was evident that a continuous damp atmosphere was not an essential factor for infection, providing the conidia reached the host in the presence of water, and that the fungus, once established, could persist over a period of extremely dry weather, and resume activity as soon as damp conditions again prevailed.

Whilst the ears of the control plants continued development and formed grain, those inoculated made no further progress; the ears inoculated at flowering time yielded no grains at all, whilst those inoculated

after flowering bore only rudimentary grains covered with mycelial growth and frequently with the "mucous" conidial masses, these fungal growths binding together the glumes and pales of many spikelets. The ears inoculated but not covered gave rather different results, grain production being irregular. Most of the florets infected at flowering time bore no grains, whilst many of those infected after flowering produced comparatively large, but shrivelled grains. In these latter the embryo was shrunken and dead, as a rule. These results appeared to arise from infection at the point of attachment of the spikelet to the rachis, the development of the grain being checked at a somewhat later stage, and the embryo killed or its development inhibited, according to the rate of progress of the parasite. The results following inoculation without covering closely resembled those following natural infection in regard to invasion of the grain from the base of the spikelet. There is, however, another, and equally common mode of natural infection in the later stages of growth, the fungus attacking and penetrating the glume, and invading the pericarp, where it causes a diffuse, brownish patch on the anterior side of the grain. Such grains may also become more or less shrivelled according to the progress of the fungus within them.

(3) *Natural Infection in the Field.*

Removal of the covers from inoculated ears and leaving the plants standing in the wheat field was equivalent to establishing centres of disease in that crop. All inoculated ears, except 12, were exposed by July 19th, which was the first day of a wet period, the rainfall (3·80 in.) during the ensuing 10 days being such as to bring the record for that month to the highest (with one exception) at that centre for 20 years[1]. During this period the disease spread alarmingly from these centres of infection in the direction of the prevailing winds to the far side of the field, the number of affected plants and the severity of attack diminishing in proportion to the distance. In the vicinity of the inoculation centres the damage was so severe, "laid" patches appearing brown as if rotting, that it was decided to cut out and burn these parts for the benefit of the rest of the crop. This proved unnecessary, for on the 29th the weather changed suddenly to almost continuous daily sunshine, and no further rain fell until the crop was stacked. The change arrested the further extension of the disease almost completely, and when the plants were dry on the second fine day, a casual observer would scarcely have noticed the presence of the disease except in the badly affected centres

[1] Average rainfall at that centre for the month of July, 2·12 in.

in the headlands. These patches were cut and removed separately at harvest time.

Specimens of these *naturally* infected ears were examined, and showed striking results as regards yield of grain, and germination capacity of the grain, as recorded below:

	Healthy ears from crop	Ears from neighbourhood of *F. culmorum* inoculation	Ears from neighbourhood of *F. avenaceum* inoculation
No. of grains per ear; average of 10 representative ears	57·6	35·6	39·6
Weight in gm. of 1000 grains taken in the recognised way	47·8	15·2	33·1
Germination capacity of sample	89 %	35 %	49 %

The smaller number of grains per ear shows that there was some early casual infection, which prevented grain formation as was the case under inoculation. The weight of equal numbers of grains was reduced by approximately one-third the normal weight where *F. avenaceum* prevailed, and by two-thirds where *F. culmorum* prevailed, but though this loss is so serious, in practice it would be even greater, because much small grain here included would be lost in threshing with the chaff and "tail" corn. Taking into consideration all these facts, reduction in number of grains, of size and weight of those formed, and loss of small ones in threshing, it can be understood why an affected crop may give only one-quarter to one-half of a normal yield, and why this phase of the disease is spoken of as "blight" of the ears.

During the germination tests it was observed that grains which showed sunken, brown (presumably dead) embryos gave no seedlings, but became covered with one or other of the two species of Fusarium in question; other grains which showed a discoloration of the side or apex gave seedlings which, in most instances, became attacked by the fungus as in the "Abnormal Conditions" experiment. Therefore seed corn from a crop attacked in the ears by either of these species of Fusarium may not only show a germination capacity reduced by nearly one-third or one-half as stated in the table, but the germination figure obtained will not represent the probable "stand" because of the subsequent death of many seedlings.

The figures in the above table indicate that, under the same conditions, *F. culmorum* became more prevalent than *F. avenaceum*, and microscopical examination of affected parts of culms and ears taken at random throughout the crop verified this. Both species were found on all aerial parts of the varieties Rector, Victor, Yeoman II, Iron I, and

Iron III, but the exceptionally dry weather during the four weeks preceding the carrying of the crop, together with the situation of the disease centres in relation to the rest of the crop, precluded a satisfactory investigation of the relative susceptibility of the wheats named.

Cultural Characters and Nomenclature.

Cultural characters of F. culmorum (*Text-fig.* 1).

Aerial mycelium; abundant, often 1 cm. high, from original material and non-normal cultures; less abundant on slants inoculated from sporodochia.

Wheatmeal agar, and hard oat agar: white, with yellow, rose pink, and carmine patches; mats down to carmine, Eugenia red, and finally brownish shades[1].

Hard potato-dextrose agar: white, with yellow, rose, and carmine patches; mats down to Pompeian red and shades of brown.

Salts-dextrose agar: white, with yellow, rose pink, and carmine patches; mats down to Indian lake.

Potato (raw) plug: white, rose, finally orange-brown, with carmine patches.

Wheat grain: white, with interspersed yellow and light carmine, matting to a light brick red covering.

Rice: white, with buff, begonia rose, and light carmine; mat is a mixture of these in diffuse patches.

Medium:

Wheatmeal agar, and hard oat agar: plectenchyma carmine to ox-blood red; deeper parts brick red to shades of brown.

Hard potato-dextrose agar: chrome-yellow to carmine, then reddish brown; deeper parts eventually burnt lake.

Salts-dextrose agar: plectenchyma carmine and remaining so; deeper parts shading to vinaceous-purple or plum.

Wheat grain: coats converted to reddish brown substratum.

Sporodochia; when first appearing in cultures, from original material or non-normal cultures, small on most media, large on a few; developed in or on the plectenchymatic layer, and continuing growth for months.

Wheatmeal agar: few, eventually up to 5 mm. diameter; honey yellow to Mars yellow, cinnamon-brown, finally Sudan brown.

Hard oat agar: numerous, average 2 mm. diameter; ochre-yellow to orange chrome, orange-rufous; later mahogany red to Sudan brown.

Hard potato-dextrose agar: few, some 2 mm. diameter; ochraceous-brown, cinnamon, to Sudan brown.

Salts-dextrose agar: numerous, minute, tending to clusters up to 5 mm. diameter; apricot orange, coral red, Mars yellow to ox-blood red (or maroon on saccharose medium).

Potato (raw) plug: few, large, up to 5 mm. diameter; honey yellow to orange-brown, then cinnamon to Sudan brown.

Wheat grain: usually filling the interior of the grains, and occurring between them; carmine to ox-blood red.

[1] Ridgway's "Color Standards and Nomenclature."

Rice grain: none obtained up to the present.

Pionnotes; numerous closely aggregated small sporodochia (*i.e.* Sherbakoff's pseudopionnotes) developed freely on slants inoculated from sporodochia, forming a continuous layer.

Wheatmeal, hard oat, and hard potato-dextrose agars: ochre-yellow, orange-cinnamon, to cinnamon or Sudan brown.

Salts-dextrose agar: apricot, apricot orange, coral red, Pompeian red, to Sudan brown.

Potato agar (high acidity): finally maroon to Pompeian purple.

Chlamydospores; quite common on wheatmeal, oat, and potato agar media, and in the dead tissues of artificially infected seedlings; conidial forms, occupying 1 to 4 (usually 3 middle) segments, up to 14·5 μ diameter; mycelial forms, sometimes intercalary and small, 8·5 μ diameter, more frequently terminal and 8·7 to 13·8 μ diameter, singly or in chains or clusters. No distinct sclerotial bodies have been observed.

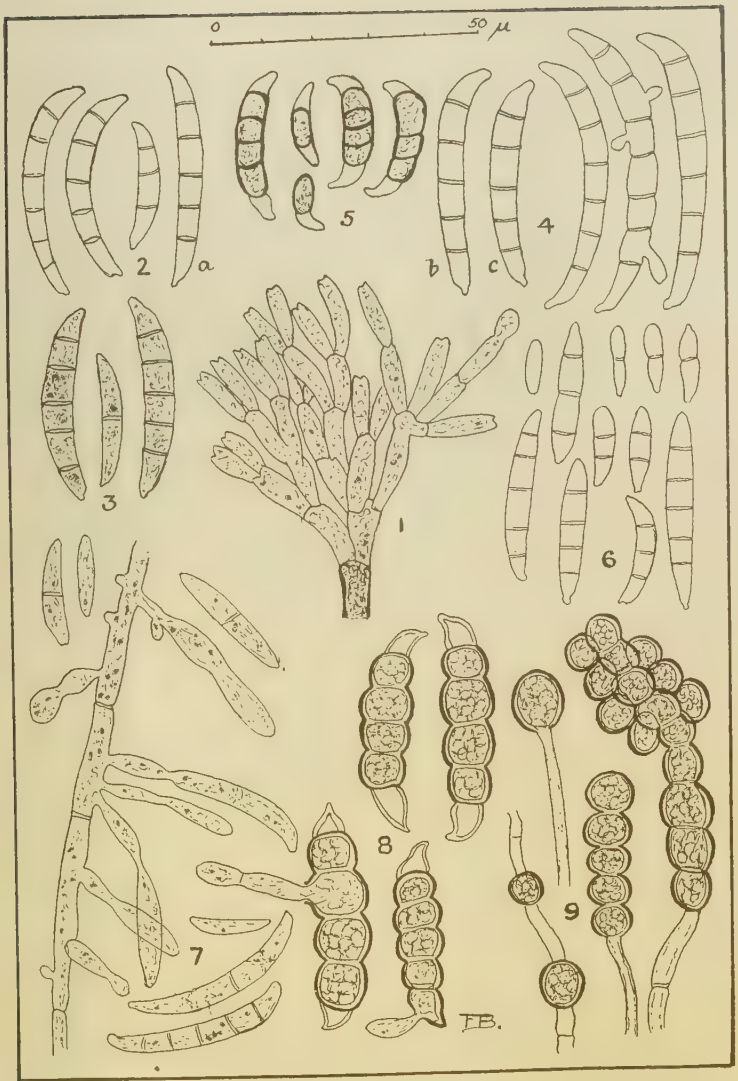
Microconidia; abundant on aerial mycelium and frequent in pionnotes of some media, mainly egg-shaped, oblong or reniform, to spindle- and sickle-shaped.

3-septate forms, generally indistinguishable from macroconidia;
0-septate, 6 to 9 μ × 3 μ; 1- and 2-septate, 9 to 20 μ × 3·5 μ;
3-septate average 26·0 μ × 4·3 μ.

Macroconidia; sickle-shaped, widest at one-third to one-half the distance from the apex; the apical cell sometimes constricted near the extremity; distinctly pedicillate in well-developed fresh stages; walls comparatively thick and septa very pronounced; ochraceous-orange tinge in mass; typically 5-septate; on aerial mycelium as well as in sporodochia and pionnotes.

5-septate: sporodochial; these predominate, sometimes up to 75 per cent.;
on wheatmeal and hard oat agars up to 60 per cent., measuring from 37·5 × 5·8 μ and 40·6 × 4·3 μ to 52·2 × 6·5 μ; average 40·6 × 6·0 μ;
on hard potato-dextrose agar, up to 40 per cent. of the total, 35 to 48 × 5·5 to 5·8 μ; average 40·6 × 5·7 μ;
on salts-dextrose agar up to 70 per cent. of the spores; 36·5 to 52·0 × 4·5 to 6·8 μ; average 42·5 × 5·9 μ;
mycelial; on seedling wheat varying from 5 to 50 per cent.; 38·5 to 47·5 × 5·8 to 6·0 μ;
average from all sources 40 to 50 × 5·8 to 6·0 μ.
4-septate: sporodochial; average from all media 38·5 × 6·2 μ.
3-septate: sporodochial; average from all media 29·5 × 5·2 μ.
6-septate: rare in culture, common on natural materials; average size 52·2 × 5·8 μ.
7- and 8-septate: very rare in culture, occasional in nature; 49·8 to 61·0 × 6·1 to 7·0 μ; average 55·6 × 6·3 μ, the majority being of the same size as the larger 6-septate conidia, with one, two, or even three additional septa.

The characters detailed show the fungus to be *Fusarium culmorum* (W. G. Sm.) Sacc., Syll. Fung. 11, 651 (= *F. rubiginosum* App. and Wr. 1910, and *F. versicolor* Sacc., Syll. Fung. 16, 1099). Considerable attention was given to comparison with *F. culmorum* var. *leteius* Sherb. (17)



Text-fig. 1. *Fusarium culmorum* (W. G. Sm.) Sacc.

1. Sporodochial elements.
2. Conidia, sporodochial, from oat agar.
3. Macroconidia, abnormally wide, from source as Fig. 2.
4. Macroconidia, including largest forms, from wheat seedling after inoculation.
5. Conidia from culture one year old.
6. Microconidia; some of the varied forms.
7. Conidial formation on aerial mycelium on incubated seedling.
8. Chlamydospores, conidial, from wheat agar (above) and germinating in water (below).
9. Mycelial chlamydospores and sclerotial cluster from wheat grain.

(*a*, *b*, *c* are the typical forms of macroconidia.)

on account of the relatively few and large sporodochia which marked the transition to the normal stage of culture; the diagnostic characters, however, ruled out this variety, and the species as named was verified by Dr Wollenweber[1].

This fungus was first described by W. G. Smith (18), under the name of *Fusisporium culmorum*, as occurring on wheat, forming pale, yellow-orange gelatinous masses on parts of the ear, and giving a spurious appearance of ripeness. No further study of this fungus has been made in this country, apparently, although its association with other phases of the disease has been suspected. It must now be recognised as an important pathogen on wheat, barley, oats and rye, causing "seedling blight" and "foot-rot," with the subsequent developments termed "thinning out" and "deaf ears," in addition to its parasitism on the culms and ears. This species is more frequent on the aerial parts than is *F. avenaceum*, probably because it is better suited by a higher temperature and drier atmosphere than the latter, for Wollenweber (21) states that "it causes heavy losses in parts of U.S.A. having an average temperature five degrees higher than middle Europe." *F. culmorum* has been recorded in most European countries, but most of the older Continental investigations concerning Fusarium are unreliable, owing to the difficulty of diagnosing species prior to the comparatively recent work of Appel and Wollenweber in Germany, and Sherbakoff in U.S.A.[2]. Schaffnit (15) recognised *F. culmorum* in association with the more common *F. nivale* (*Calonectria graminicola*) the cause of "snow-mould" in Germany, but did not investigate the activities of the species. In U.S.A. Wollenweber (21) reported it a serious enemy of seedlings of cereals, causing damping off of wheat and oats and to less extent barley, whilst Atanasoff (4) stated that it seldom causes blighting of wheat and rye heads in the wheat-growing region of U.S.A., whereas in Holland it is the common cause of this trouble. In France, according to Foëx (10), it causes foot-rot of oats and barley. McAlpine (13) records *F. culmorum* in Australia.

The host plants mentioned in the various records are: Triticum, Secale, Hordeum, some grasses as Agropyrum, Bromus, etc., Lupinus, Beta, Solanum and Cucurbita. The host range, in addition to that of the four cereals, remains to be determined for this country.

[1] Correspondence of May 1925 is gratefully acknowledged.

[2] For this reason references to *identified F. culmorum* and *F. avenaceum* only are quoted in this paper.

Cultural characters of F. avenaceum (*Text-fig.* 2).

Aerial mycelium; generally abundant from original material, and non-normal cultures, less or nearly absent on slants inoculated from sporodochia.

Wheatmeal agar: fluffy, white, with traces of rose pink, on a dense, white felt; mats down to a white felt on a carmine substratum.

Hard oat agar: as on wheatmeal agar, but distinctly less abundant.

Hard potato-dextrose agar: abundant, fluffy, white, with transient traces of rose and yellow.

Salts-dextrose agar: white, with traces of rose pink or carmine, matting to a white felt with carmine patches.

Potato (raw) plug: white, very abundant; mats to a thick, white felt.

Wheat, rice grains: white with traces of rose and yellow; when old brownish red near the grains.

Medium:

Wheatmeal and hard oat agars: plectenchyma carmine to ox-blood red; deeper layers Bordeaux.

Hard potato-dextrose agar: plectenchyma yellowish then carmine, ox-blood red, and Bordeaux; deeper layers eventually shades of brown.

Salts-dextrose agar: plectenchyma carmine, passing in deeper layers to pomegranate purple.

Wheat grain: surface of grains shades of yellow, and finally buckthorn brown.

Rice: surfaces of grains maize yellow, then orange-yellow, but masked by the mycelial felt.

Sporodochia; when first appearing in cultures from original material or from non-normal cultures, typically few and large; they arise in or on the plectenchymatic layer and continue to increase in size for some months.

Wheatmeal and hard oat agars: appeared first when cultures were 3 to 4 weeks old, and production continued up to 6 months; at 3 months the sizes ranged from 1 to 6 mm. in diameter; at first apricot yellow then apricot buff; when old ochraceous-orange to cinnamon-rufous.

Hard potato-dextrose agar: fewer and smaller than on oat and wheatmeal; apricot, apricot buff, rose buff, to vinaceous-rufous when old.

Salts-dextrose agar: usually in clusters of two or three which coalesced to single ones 3 to 5 or 6 mm. diameter; apricot, apricot buff to apricot orange.

Potato (raw) plug: few, large, up to 6 or 8 mm. diameter; apricot, apricot buff.

Wheat grains: as on potato, but eventually apricot orange.

Rice grains: few, large, 3 to 6 mm.; apricot to vinaceous-rufous.

Pionnotes; formed on most media inoculated from sporodochia, as an almost continuous layer of small sporodochia (pseudo-pionnotes) covering the plectenchymatic layer in the surface of the medium. Strongly acid potato-dextrose agar has so far proved an exception. On moist media the sporodochial layer is masked by an overlying mycelial felt. Its colour, as a rule, resembles that of the plectenchyma.

Wheatmeal agar: at 5 to 6 months apricot orange, slimy on the deep carmine plectenchyma, and covered with a thin mycelial felt.

Hard oat agar: less extensive than on moist media, and much less aerial mycelium; colour remains honey yellow to apricot.

Salts-dextrose agar: somewhat variable between wheat and oat forms.

Chlamydospores; in the conidia pseudo-chlamydospores are particularly common as segments or cells with slightly thicker walls and denser contents, from 5·0 to 7·5 μ wide. True chlamydospores, spherical, double walled, and 7·5 to 9·0 μ in diameter, also occur occasionally, more particularly on potato media, the conidium, as a rule, containing only one such spore. Mycelial chlamydospores are terminal, generally in chains, and reach 11·6 μ in diameter; they occur in the plectenchyma of old cultures on wheatmeal and potato agar, and in the tissues of dead bases of wheat (and probably other) plants.

Sclerotia. These are formed by aggregations of segments resembling chlamydospores, except that the outer walls are scarcely so thick. They occur on potato and wheatmeal agar, appearing in the plectenchymatic layer as glossy, pale yellow structures, sometimes 1 mm. across; they are brittle, snapping under pressure, and when transferred to fresh media they resume growth readily. Similar structures occurred in the dead tissues of plant bases.

Microconidia; abundant on aerial mycelium, and sometimes the only spores produced, on natural material; so also on all culture media until the sporodochial stage is reached. Not distinguishable from the corresponding spores of *F. culmorum*.

3-septate are predominant, spindle-shaped, 23 to 27·5 × 4·3 to 5·5 μ; 3-septate spores similar in form to macroconidia are common.

0-septate, elliptic, oblong, to fusoid, 11·6 to 20·3 × 3·0 to 3·5 μ.

1-septate, fusoid to spindle-shaped, intermediate in size between 0- and 3-septate; 2-septate, similar but very rare.

Macroconidia; slender, elliptically curved, frequently straight for the greater part of the length then sharply curved at the upper end; nearly uniform in width throughout, narrowing gradually at both ends, the lower end rather more blunt than the apex; sub-pedicillate, the point of attachment slightly lateral, but discernible only when freshly detached; walls and septa, especially the latter, extremely thin. Macroconidia are borne on hyphae of aerial mycelium, in sporodochia, and pionnotes; the spore-bearing branches of sporodochia closely resemble spores.

5-septate: sporodochial; these predominate, sometimes up to 95 per cent.;

from wheatmeal, hard oat, and salts-dextrose agars the sizes are similar, 43·5 × 4·0 μ to 69·7 × 4·2 μ; average 58·2 × 3·9 μ; slightly smaller from hard potato-dextrose agar, average 54·5 × 4·0 μ;

mycelial; from hard potato-dextrose agar, 43·1 to 65·9 × 3·5 to 3·6 μ; average 57·0 × 3·5 μ;

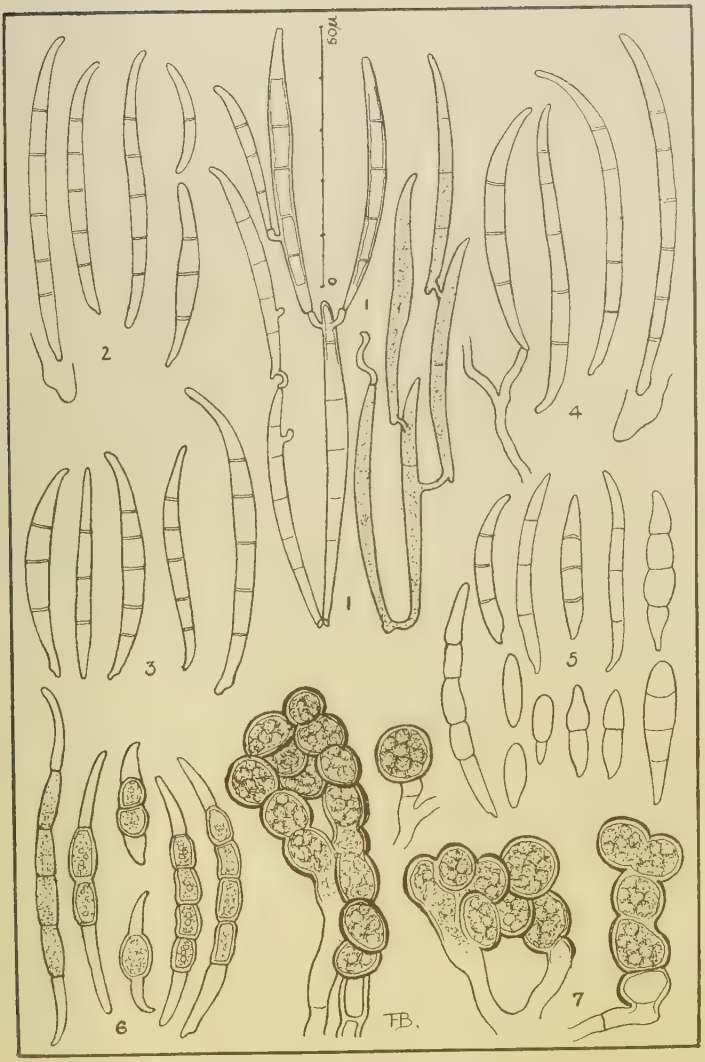
from seedlings, 43·7 to 52·2 × 2·9 to 3·2 μ;

from field wheat, 52·2 × 3·0 μ; 58·0 × 3·5 μ; 60·9 × 4·0 μ; 75·0 × 3·8 μ; 85·0 × 3·8 μ; average on wheat 59·5 × 3·7 μ;

mycelial spores are narrower and longer than sporodochial.

3-septate: sporodochial; 37·7 × 5·5 μ to 43·5 × 5·1 μ, becoming narrower as they elongate.

6-septate: frequent on mycelium on vegetable tissues, rarer in sporodochia; average 65·8 × 4·0 μ.



Text-fig. 2. *Fusarium avenaceum* (Fries.) Sacc.

1. Sporodochial elements.
2. Conidia, sporodochial, from oat agar.
3. Conidia, sporodochial, from salts-dextrose agar.
4. Macroconidia from aerial mycelium, on seedlings.
5. Microconidia from aerial mycelium, various sources.
6. Pseudochlamydospores from mature and old cultures.
7. Chlamydospores and sclerotial bodies from seedlings (left) and potato agar culture (right).

7- and 8-septate: occasional on vegetable tissues, but rarely larger than the 6-septate spores.

0-, 1- and 3-septate spores, the last approximately equal in size to the shorter 5-septate ones, all immature forms of the larger typical spores, occur in sporodochia and on aerial mycelium.

No spores with more than eight septa have been observed.

The characters detailed above show the fungus to be *Fusarium avenaceum* (Fries.) Sacc. (= *F. roseum* Lk. var. *lupini albi* Sacc. in Rabenhorst's *Kryptogamen Flora*, IX, and *F. subulatum* App. and Wr.(21)). The species shows the presence of chlamydospores and sclerotia, structures not previously recorded for it. The occurrence of chlamydospores, together with certain morphological characters, bring the species very close to *F. arthrosporioides* Sherb.[1], but it differs in other essentials such as size and colour of sporodochia, the 5-septate sporodochial conidia on hard oat agar, absence of pyriform microconidia, and coloration of hard potato-dextrose agar. The only other species with which it may be confused is *F. herbarum* (Cda.) Fries. (= *F. metachroum* App. and Wr.), from which it differs in average size of 5-septate conidia from sporodochia, coloration of medium, and relative abundance of 6- (or more) septate conidia in culture and in nature.

F. avenaceum has not, apparently, been described in relation to a disease of cereals in Britain. It must now be recognised as a pathogen on wheat, barley, oats and rye, more frequently as a cause of "seedling blight" and "foot-rot," the latter phase resulting in "thinning out" and "deaf ears"; it occurs also on the aerial parts, especially the ears. A probable reason for its greater frequency as a foot-rot organism and its rarer occurrence as a cause of ear blight, as compared with *F. culmorum*, has been mentioned on p. 228, and in this connection it is of interest to note that, according to Naumov(14), *F. avenaceum* occurs in northern Russia as the common and almost sole cause of blighting of cereal heads. Whilst Atanasoff(4) states that "its geographic distribution as pathogene is limited to Northern Russia, but it has been observed in a number of cases in Holland and N. Wisconsin (U.S.A.)," Schaffnit(15) and others, *e.g.* Appel and Fuchs, mention its occurrence in central Europe, and the present writer finds it extremely common in northern England. The various records mention this species as occurring on wheat, spelt, oats, barley, rye, maize, Solanaceae, Chenopodiaceae, Leguminosae, Carex, and seedling willow, beech and laburnum.

[1] Sherbakoff's diagnosis of *F. arthrosporioides* stated that it had no true chlamydospores. Atanasoff (1923) showed that under suitable conditions the species produced such spores singly, in chains, and in clusters.

Physiological Studies of *F. culmorum* and *F. avenaceum*.

The studies here recorded are those having a direct bearing upon the practical aspects of the Fusarium disease of cereals, and yielding information necessary for the elucidation of the disease cycle and for indicating methods of control. A point of considerable importance in this respect is the presence or absence of an ascigerous stage, to produce which many and varied attempts have been made, starting from the point advised by Wollenweber(21). Both single and mixed conidial forms were grown on a wide range of artificial media, under various conditions of temperature, light, and moisture, and on such natural material as seedlings, culms and ears, mature and immature, in laboratory, greenhouse and field. That no signs of an ascigerous stage could be found under any of these conditions indicates that these two species of Fusarium are amongst the many which have no ascigerous stage, and supports the statement of Wollenweber(21) that "we have no conclusive proof that Fusarium is the obligate conidial stage of Ascomycetes."

Influence of Temperature. The resistance of conidia to moist heat was ascertained by making count cultures from aqueous suspensions of conidia, after subjecting the suspensions to given temperatures for 3 minutes. The results may be shown concisely as below, from which it appears that the death point for conidia of both species is about 50° C. The effect of dry heat has not been investigated.

	Av. No. of colonies per dish on 10 dishes			
Conidia	Control	At 44°–45° C.	At 49°–50° C.	At 54°–55° C.
F. culmorum	12	10	4	0
F. avenaceum	10	9	1	0

For low temperature trials cultures on cooked wheat grains were exposed out of doors for periods of 2 and 3 days with temperature range − 5° to 0° C., and others for 3 months with temperature for the greater part of the time between − 10° C. and + 5° C. Conidia from sporodochia of each species showed rapid loss of vitality, proving them to be unable to withstand winter conditions for any considerable time.

	Av. No. of colonies per dish on 10 dishes		
Conidia	Control	2 days' exposure	3 days' exposure
F. culmorum	15	12	8
F. avenaceum	13	9	4

The mycelium of these exposed cultures, on the contrary, grew intermittently throughout the period, and subsequent growth was not in the least adversely affected.

With regard to the infection of growing seedlings, Dickson (7) states that for blighting of seedlings by *Gibberella saubinetii*[1] the minimum temperature is 12° C. This certainly does not hold good for the two species of Fusarium under consideration, for diseased seedlings were produced abundantly and repeatedly in contaminated soil and from contaminated seed under glass during winter months when the temperature never reached 10° C. between planting and lifting times. As stated above, the growth of the mycelium does not cease in winter, and as infection is accomplished by mycelial hyphae as readily as by conidia, it is probable that infection in the field occurs at any minimum temperature which suffices for the growth of wheat seedlings, or at even lower temperatures when development of the fungi but not of the seedlings proceeds. It appears, therefore, that under ordinary field conditions in this country, these two fungi do not lose vigour or die out during winter, though their dissemination by means of conidia will be more or less inhibited; on the contrary, they continue to some extent their growth and attack on host plants.

Longevity of the Fungi. This feature was investigated from cultures kept at room temperature (5° to 18° C. approximately) for 12 months or longer. Micro- and macroconidia borne on aerial mycelium, and thus exposed to atmospheric desiccation, had lost vitality completely after 15 months, but conidia from still moist sporodochia (then of gummy consistency) grew freely in fresh media, and probably retain vitality much longer than this. Portions of mycelium, including chlamydospores, also gave excellent growth after 15 months; but after 3 years (the oat medium being then like parchment) the whole organism was quite dead. Some species of Fusarium, but not those under present consideration, retained vitality on cooked stem material for 8 years, according to Maneval (12). Thus, *F. culmorum* and *F. avenaceum* might be expected to retain vitality on grain, straw and refuse under storage conditions for 12 months or more, so that such infected materials would readily act as carriers of infection from one season to the next. In the soil it is probable that some new growth occurs on organic matter every season, quite apart from living plants, and that the pathogens persist in contaminated soil for several years.

[1] *G. saubinetii* (Mont.) Sacc., the conidial stage of which is *Fusarium rostratum* App. and Wr. and *F. roseum* pro parte.

Relation of Fungus to Host. In the pot experiments previously described, the "deaf ears," whether remaining within or protruded from the sheaths, were themselves free from infection, though the plants were severely affected with Fusarium at the bases. This was true also of "prematurely ripe" ears in the field, unless they had been infected casually externally. These facts led to observations as to the extent to which the fungi invaded the host plants before actual death of the latter. Invasion of the plant from externally contaminated grain[1] occurred by growth of the fungi within and without the pericarp to the primary roots and primary (wiry) stem, both of which structures became penetrated in the neighbourhood of the grain. Atanasoff (4) considers that the moribund coleorhiza and coleoptile afford a favourable basis from which such attack proceeds. The fungi proceeded up the primary stem, confined to its outermost layers until loss of vitality through age or otherwise, when all its parts became permeated; the parasite thus reached the "crown" of the seedling. Infection from contaminated soil appeared to follow a similar course, passing in both directions from the point of invasion, for infection was not found to be restricted to any particular part of the underground system. From the diseased crown the roots became affected at their proximal ends, some young roots being destroyed, and the general root system was more or less reduced. In affected older roots no tissues were free from invasion, and browning and death proceeded slowly towards the distal ends. Atanasoff (3) states that plants with rotting roots and bases, when transplanted to good soil, recovered and produced heads as normal as those of control plants. This indicates that in good, well-drained soil the vigorous new root growth is not attacked to a serious extent, and this view is supported by the development of comparatively extensive roots by all four cereals in the large pot experiments (p. 218). The extent of invasion and destruction of root tissue by Fusarium is determined mainly by the amount of soil moisture and the rate of growth of the root system.

From the crown the fungus frequently extended into the lowest aerial internode, there existing in some portions of all the tissues (Plate XII, fig. 4), the hyphae being both inter- and intra-cellular. These hyphae were aggregated in places below the epidermis, more particularly in the cavities below the stomata, and from these masses conidia-bearing hyphae were protruded to the exterior either through the stomata or

[1] Grains infected internally soon produce mycelium externally which proceeds as described; tracing the path of the fungus from the interior of such grains into the seedlings has not been attempted.

between the epidermal cells. The mycelial growth was massed in the xylem also, and the blocking of these elements and consequent restriction of supplies of water from the soil at a critical time is considered to be the direct cause of the "premature ripening" of plants about the time of shooting the ears. Some plants with rotten crowns, which remained standing and even bearing a few, shrivelled grains in the ears, did so by the aid of one or two thickened roots arising from the node just above soil level (Plate XII, fig. 5). When standing stems remain moist by partial supplies of soil moisture, aided by reduced transpiration consequent upon a moist atmosphere, they may be invaded and discoloured to some height. Hence the determining factor in stem, as in root, invasion is the amount of available moisture.

The height to which Fusarium extended in the stems of plants diseased at the base was investigated by examining the successive internodes before the plants actually died off. The results obtained from 66 pot and field plants showed that the fungi occurred frequently (41 times) in the internode next above the soil level, occasionally (15 times) in the second internode, and not once above this, under normal conditions of moisture. A number of field plants were examined, each showing a brown culm extending well up towards the ear, enclosed for the greater part of its length in bleached leaf-sheaths, with the ear bearing shrivelled grains in the bleached chaff; these grains were not infected nor did the ears show the characteristic lesions, although one or other of the species of Fusarium was obtained from any part of the brown culm. It was evident that *F. culmorum* and *F. avenaceum* do not cause infection of ears by growing up the stems from diseased parts lower down, as happens with the allied fungus *Gibberella saubinetii*, according to Doyer(8). Diseased ears and grain are due solely to infection by conidia distributed from external sources.

Certain phenomena which have been recorded in this investigation demand some further consideration. It has been shown that the fungus failed to penetrate the internodes of healthy young plants, that the tillers arising from diseased crowns remained green after the main stems were bleached and dead, and that ears produced by stems badly diseased at the bases remained free from infection. On the contrary, these two Fusarium species readily attacked all mature and maturing aerial parts. These differences can be ascribed only to the nature of the cell walls or of the cell contents at different stages of growth. The chemical nature of the cell walls of wheat seedlings grown under different conditions has been investigated by Eckerson and Dickson(9), but careful con-

sideration shows that no satisfactory explanation of the phenomena mentioned can be based on this factor. An explanation appears possible only on the basis of the nature of the cell contents, as is the case with varieties of wheat resistant to certain Rust fungi. In this case it would appear that the young cells of the meristematic and growing tissues of our varieties of cereals remain unattacked when situated on or carried through diseased areas because of the nature of their cell contents. This would afford an explanation of the phenomena mentioned, and suggests for further consideration the hypothesis that the capacity for resistance to Fusarium is a property of the cell contents of such immature tissues as exist in our present varieties of cereals, and that this property will be the determining factor of the resistance to Fusarium disease if such resistant varieties are eventually obtained.

In this connection it is of interest to note that Sherbakoff (16) suggests that *F. culmorum* not only exists as distinct varieties, but that the varieties may be composed of distinct biological strains. The two species of Fusarium dealt with in this investigation show no definite signs of specialisation or adaptation to any one of the four cereals. Variability of virulence may accompany that variability of morphological characters exhibited by this fungus, and the suggestion of biological strains must for the present be regarded as tentative only. Should the suggestion prove to be a fact, however, it would support the hypothesis advanced above.

Cycle, Symptoms and Control of the Disease.

Previous Investigations. A brief review of the Continental and American work concerning this disease will be helpful for comparative purposes. Although Fusarium has been observed and recorded on cereals for very many years, the different phases of the disease had been treated as distinct troubles both in Europe and America, and owing to the difficulty of isolating and identifying the different species prior to the standardising methods of Wollenweber, Sherbakoff, *et al.* (23), the earlier records are confusing or contradictory. The most complete and recent work, by Atanasoff (4), gives an extensive review and bibliography on this subject, from which it appears that the only investigations of the disease as a whole were as follows, and even these are open to question according to the extracts appended from the said bibliography:

Mortgensen (1911) "did not state which or how many Fusarium spp. were concerned in the conditions described."

Schaffnit (1912, 1920) "the only really scientific work on the Fusarium blight in

Europe"; dealing in detail with *F. nivale* he "committed great errors whenever he tried to treat the disease as a whole."

Naumov (1913, 1916) dealing in detail with *Gibberella saubinetii* and *F. avenaceum* as causes of "Intoxicating Bread" in Russia, "must have worked with impure cultures" and "the results obtained by him must be repeated and confirmed before being accepted."

Lindfors (1920) investigated...and *F. culmorum* in connection with foot-rot and head-blight; "difficult to understand why...did not watch his plants more closely."

This brings the record of such "complete" investigations up to the time of Atanasoff's publication in 1923, with which it appears desirable to compare notes. As Atanasoff points out, the complete life-histories of some of the Fusarium species concerned in this disease are unknown, and he presents for his purpose the life-history of *Gibberella saubinetii*. This fungus does not occur, so far as is known, in this country, and the life-cycles of our predominant species have received due attention. Atanasoff states of Fusarium species which have no known ascigerous stage, "which included the greater number of the fungi studied here, there is no experimental evidence to show how they overwinter"; it is "not decided whether the mycelium wintered in diseased stubble, etc., or that the conidia produced in the fall retained vitality." This point has been settled so far as concerns the two prevalent species in this country. Discussing the various phases of Fusarium disease, Atanasoff states that the blighting of heads is by far the most important one, that it has been generally overlooked, and that European investigators attributed undue importance to the attacks on maturing heads and kernels because "they were ignorant of the so common and purely parasitic blighting of the heads." The blighting of the heads has been known since the time of W. G. Smith (p. 228), who speaks of the spurious appearance of ripeness, and is quite common in this country, or at least the northern part of it, but no "uniform layer (pionnote) of conidia extending over a large portion of the head" occurs here even in very moist weather. An equally important phase, from an economic point of view, is the "chronic" foot-rot, which results in small, shrivelled grains in the ears, or in completely "deaf ears"; this latter aspect is not mentioned by Atanasoff, and would appear to be of little importance elsewhere, although in 1908 Appel(1) stated that cereal plants infected basally with Fusarium "later appear exactly like plants attacked by the foot-rot disease as due to Ophiobolus, etc." The cycle and symptoms of the disease described below agree in correlated phases and effects with the general account given by Atanasoff (*l.c.*), but the description is confined to the summarising of the experimental and field observations

recorded in the foregoing pages, using the popular names for the successive phases, so rendering it applicable to our "regional" conditions.

Origin of Cycle. The two common species of Fusarium which attack wheat, *F. culmorum* and *F. avenaceum*, may be present in the first instance with the grain sown or in the soil. With the grain the fungi may occur adhering externally, in which case they are killed by the "pickling" processes usually practised; but more frequently the fungi are actually inside the grain, either in the embryo which is then usually dead, or in the pericarp which is marked by a slight brownish discoloration, or in the endosperm when the grain is more or less shrivelled. The mycelium may be present in any or all of these parts, and can be observed (best by differential staining) under the microscope. Grains with dead embryos do not germinate, whilst those affected otherwise yield seedlings which are very liable to basal infection. The fungi persist in the soil on diseased residues from the previous season, but how long they exist there has not been ascertained; they certainly accumulate when susceptible crops like cereals are grown frequently. They are not killed out by any winter cultivations, and their vegetative structures are capable of withstanding our lowest natural temperatures, and have been found in active growth on diseased residues in spring after exposure in the field throughout a severe winter.

Seedling Blight. The seedlings are attacked at the base by the fungi either from the grain itself or from the soil as described on p. 216. They show a browning at the bottom of the stems and on adjacent parts of the roots. Such seedlings may wilt and die off after brairding, this "seedling blight" resulting in a thin stand. In a wet soil and damp atmosphere the fungi emerge from the still living stems of seedlings at soil level, and extend somewhat on the soil around the plant, this corresponding to the "snow-mould" which prevails on the Continent. This mycelial growth around living plants and overgrowing dead ones, during both autumn and spring produces conidia which are distributed to neighbouring plants and cause further basal infections. Thus a single diseased grain or seedling may act as a centre of infection for some distance around itself.

Spring Yellows. Many affected seedlings continue their existence as young plants diseased at the bases. As the fungus continues to increase within the tissues during mild periods in winter, these plants in spring make but slow growth, the foliage is of a yellowish green colour, and the leaves wither from the tips downwards (Plate XII, fig. 1). The common practice of top-dressing a crop in this state with a nitrogenous fertiliser

is to be recommended, for it stimulates the production of new roots which, in a dry season, may suffice to bring the plants to maturity; in a wet season, however, which favours the progress of the parasite, the crop may remain a partial failure. Both results have been observed in field practice.

Foot-rot. This term is applied to affected plants when older, and showing a discoloured and rotting part at and below soil level. In a dry atmosphere the fungus remains restricted to these parts. The root system is generally deficient (Plate XII, fig. 1), and the existing roots brownish, whilst the soil tends to adhere to them as though attached by some exudate. The discoloration usually extends slightly above soil level, but is not readily detected in field plants. In fact, this phase is best indicated by the former and following phases.

Thinning out; Deaf Ears. When "foot-rot" plants shoot the ears the tops are frequently too heavy for the rotting bases to support, and many then fall against or between the others, thus causing the "thinning out" observed by farmers between earing time and harvest. The plants may break off naturally, or the stems come away readily if pulled, often severing at the basal node with a clean, convex fracture (Plate XII, fig. 5). Above the fracture there may be one or more thickened roots, especially after top-dressing a crop, but these often fail to carry the plants to the grain stage. Affected plants may occur singly or in small groups, and one or several shoots of a single plant may be attacked whilst its other shoots remain normal. Whether falling over or remaining erect most of the "foot-rot" plants whiten rapidly after heading out, and form the "prematurely ripe" plants or "whiteheads," with ears termed "deaf ears" because they contain little or no grain. The thinning-out and deaf-ear phases, natural consequences of the slowly developing foot-rot, are the most obvious phases of the disease, and in dry seasons the most important ones also. Sometimes plants have brown, soft culms within the leaf-sheaths, and unbleached ears bearing small, shrivelled grains; such symptoms usually follow secondary basal infections in a moist season.

Blight of Ears. The conidia produced on the bases of living plants and on infected dead matter readily cause infection in a damp season of all fully grown aerial parts, where further conidia are produced either on mycelial growth or as the so-called "mucous mould," the latter often seen on the nodes of the straw. The ears are particularly susceptible to attack from flowering time onwards, infection being marked in wet weather as a diffuse brown discoloration, and in dry weather as a

bleaching with very faint brownish markings. According to the time of infection and the situation of the point of infection, the result may be non-production of grain in one or more florets, or discoloured, shrivelled, or non-vital grain; frequently all these results occur together in varying degrees in a single ear. In a dry season the production of conidia, and the amount of infection, are very much restricted, and there is correspondingly less ear blight, but in wet seasons infection is abundant and the yield of threshed grain is much reduced. Further, according to Tomasski(19), there is a heavy loss of nutrient material (starch and protein) in infected grain both before and after harvest and when stored after threshing.

Completion of Cycle. Infected grain sown as seed, or diseased plant parts which remain in the same or neighbouring fields, serve to introduce the disease into a cereal crop for the following season. The disease may also probably arise from such grasses as couch and brome, which are believed to support both the species of Fusarium concerned.

Control of the Disease. Since the disease may be initiated by affected seed or by contaminated soil these factors demand consideration. Seed treatment would consist of some comparatively straightforward method of external disinfection were Fusarium disease carried on the exterior only of the grains, and that the earlier investigators (the most prominent being Hiltner(11) and his collaborators) practised such treatment probably accounts for the uncertainty and variable success of their methods. It is now recognised that Fusarium infection is generally located within the grain, and therefore beyond the reach of chemical disinfectants applied externally. So far as is known, application of heat is the only way of destroying such infective matter. The "hot water" method, which has proved efficacious for some seed-borne diseases, was explored and shown to be unsuccessful for Fusarium disease by Westerdijk(20). Naumov(14), after failure with all the usually practised seed treatments, tried the method of "dry heat" and found it successful for eliminating *Gibberella saubinetii* and Fusarium spp. Atanasoff and Johnson(5), failed to verify his results for times and temperatures, and themselves found the best control by heating the seed at 100° C. for 30 hours. Their report stated that "these data point only to the possibility of eliminating seed infection"; this possibility, however, is remote, since in the two samples of Fusarium-infected wheat which were tested the germination capacity was reduced by approximately 50 per cent. This method of control of the seed-borne disease, in the present state of knowledge, is obviously not a practicable or economic proposition.

Elimination of the disease from the soil by means of soil dressings has not been investigated, but it offers little prospect of economic utility. As shown experimentally the fungi thrive under such alkaline or acid conditions as would be found in ordinary soils, and the disease in this country is found, in fact, on different types of soil, ranging in condition from very good to very poor, and with and without a "lime requirement." It is much more prevalent, however, on badly drained soils (though not confined thereto), which favour the seedling blight and foot-rot phases; therefore drainage, directed especially towards prevention of superfluous or stagnant water near soil level, would prove beneficial. Improved physical condition of the soil, as favoured by well-prepared seed-beds, fallowing, and fallow crops, giving stronger and better rooted plants, would also be helpful. These are the only measures concerning soil treatment which can be recommended at present.

Rotation of crops is probably the most convenient and effective method applicable by the farmer for combating the disease. It was recorded in U.S.A. as long ago as 1899 that "Fusarium-infested soils become worthless for growing plants subject to attacks by these organisms for many years," and numerous investigators since that time have recommended rotation of crops. In this country the disease occurs under a variety of methods of cropping, but, in the author's experience, it was worst where wheat was grown every fourth year; further, it affects all four cereals, including all the varieties of wheat grown, but the loss in wheat is greater than in oat crops. Rotations in which straw crops are widely separated by others, with wheat separated still further by taking oats as an alternative straw crop, would on the more badly affected lands go far to reduce the damage to a minimum. But while this course would certainly tend to prevent accumulation of the pathogens, it would scarcely suffice to eliminate the disease from the land.

Some suggestions concerning the seed, though perhaps obvious, may be added. High grade seed, owing to its capacity for producing more vigorous plants, will give better results in contaminated soil than will poor seed. Seed corn should come from crops free from this disease to avoid infected seed, and "pickling" with formaldehyde or copper sulphate solutions will prove useful for eliminating external fungal growth, if the seed is not afterwards recontaminated from sacks or implements. For reasons already indicated prompt threshing and disposal of grain from a badly affected crop is advisable.

Summary.

Fusarium disease of wheat, common in the north of England, is due mainly to *F. culmorum* (W. G. Sm.) Sacc. and *F. avenaceum* (Fries.) Sacc. occurring either separately or together.

Both species cause "seedling blight," "spring yellows," and "foot-rot"; the foot-rot results in "thinning out" between earing and harvest, and in "premature ripening" or "whiteheads" with "deaf ears."

Both species cause a "blight of the ears" by casual external infection, resulting in sterility of florets, or diseased grain; *F. culmorum* is the more frequent cause of this phase.

Barley, oats and rye show corresponding phases of the disease.

The pathogens overwinter in diseased material in the granary or in the field; under storage conditions they retain vitality for considerably more than one year.

The cultural and diagnostic characters of the fungi are given, and control measures are discussed.

The writer is indebted to Mr F. T. Brooks for advice on the preparation of this paper.

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EXPLANATION OF PLATES XII AND XIII.

Fig. 1. Wheat from an affected crop in spring, showing the withered leaf-tips and deficient root systems.

Fig. 2. Barley seedlings; two from controls; others from soil inoculated with *F. culmorum* (4 above) and *F. avenaceum* (3 below).

Fig. 3. Rye; soil and seed inoculation with *F. culmorum.*

Fig. 4. T.S. basal internode of young wheat plant from seed contaminated with *F. culmorum.* *Xy.* = xylem becoming blocked; *St.* = stoma and cavity packed with mycelium.

Fig. 5. Bases of prematurely "ripe" wheat, showing point of fracture, and deficient root system.

Fig. 6. Effect of *F. culmorum* on wheat grown under normal conditions; inoculations—soil on left, seed on right. Inoculations with *F. avenaceum* gave similar results.

Fig. 7. Oats grown under normal conditions in soil inoculated with *F. culmorum* (left) and *F. avenaceum* (right). Barley and rye, under similar conditions, gave equally striking results.

(*Received October 8th*, 1927.)

Fig. 1.

Fig. 2.

Fig. 5.

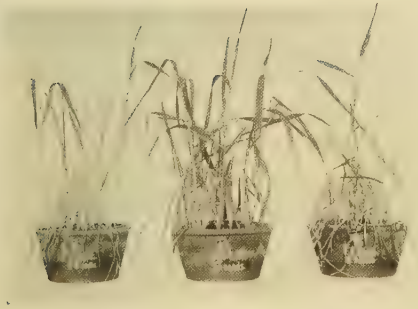

Fig. 3.

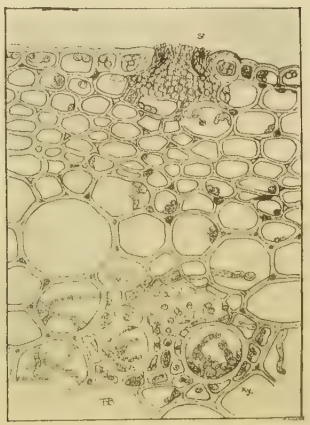

Fig. 4.

BENNETT.—On Two Species of Fusarium (pp. 213–244).

Fig. 6.

Fig. 7.

BENNETT.—On Two Species of Fusarium (pp. 213–244).

ATTEMPTS TO CONTROL BUNT (*TILLETIA TRITICI*, WINT.) IN WHEAT WITH A FORMALIN-GYPSUM DUST

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Introduction.

It is generally admitted that there are certain disadvantages in the wet treatment of seed wheat for the prevention of Bunt which might be overcome by the use of an efficient dry powder. From the practical point of view, it would be more convenient and less risky if the farmer could treat his seed wheat at any time and put it back into the sacks immediately, instead of having to do this as soon before sowing as possible. When using the wet methods of treatment, the farmer is advised to be very careful to see that the grain is quite dry after treatment and to sow as soon as possible. Using a dry powder or dust, he could treat it at any time, bag it immediately and sow at any time without fear that prolonged delays might cause a reduction in the germination capacity and consequently a thin plant in the field.

Preparation of the formalin-gypsum dust.

As the wet formalin treatment has proved to be an efficient control of Bunt in wheat, it was thought advisable to try to make a *dry powder* incorporating *formaldehyde* as the fungicidal agent.

Large quantities of a calcined gypsum powder, which is sufficiently fine to pass a "120 mesh" sieve, were available from neighbouring gypsum pits, and a formalin-gypsum powder was successfully made.

The material was prepared from 40 per cent. formaldehyde solution and calcined gypsum powder. A weighed quantity of the gypsum having been placed in a mortar, the formaldehyde solution was run in slowly from a burette during mixing. Finally the mixture was passed through a "60 mesh" sieve as quickly as possible and placed in an airtight tin.

Subsequent analytical estimates of the formaldehyde strength of the powder proved that only slight loss in strength occurred during mixing.

The following strengths were made up:

A. 1 part 40 % formaldehyde in 70 parts gypsum (by weight)
B. 1 ,, ,, 35 ,, ,,
C. 1 ,, ,, 20 ,, ,,
D. 1 ,, ,, 10 ,, ,,

Strength D would be equal to 1 pint of 40 per cent. formaldehyde to 13½ lb. of gypsum. Stronger mixtures were tried but such mixtures were too damp for practical purposes and did not adhere to the wheat grains.

Preliminary tests showed that 1 gm. of calcined gypsum powder, when well shaken with 100 gm. of wheat, left only a small portion of the powder in the bottom of the vessel. At this rate, about 2½ lb. of powder would be required to treat a sack of wheat.

Rate of infection with bunt spores.

In the first two years the only seed available was clean, so that it was necessary to infect it artificially with bunt spores. This was done by crushing some bunted grains (obtained from a crop of a different variety of wheat) into a powder and thoroughly shaking it with the wheat. The result was a very heavily infected sample of wheat of a dirty brown colour. The maximum amount of bunt spores that would adhere to the grain was used, and it was calculated that this amount was approximately equal to 1 part by weight of bunt spores to 200 parts by weight of wheat.

Method of seed treatment.

The heavily infected wheat was put into a tin box with the necessary amount of formalin-gypsum powder to cover the wheat grains, that is 1 gm. of powder to every 100 gm. of wheat. The box was closed and the mixture thoroughly shaken for a few minutes. The treated wheat was then put into small canvas bags and tied up until ready for sowing. Care was taken to avoid contamination with bunt spores after the wheat had been treated.

Germination tests.

In order to see whether the treatment had affected the germination capacity of the seed, germination tests were carried out in the laboratory at intervals for 2 months after treatment. These showed that no harm

had been done by the seed treatment and the plant produced in the field later confirmed this.

The experimental plots in the field throughout the 4 years were about 12 yards long by 1 yard wide.

The percentage of bunted ears in each plot was obtained from counts of about 1000 ears taken at random and an ear was considered bunted, even if only a few grains showed the disease.

First year's experiments—1924.

In the first year's experiments, only two strengths of powder were used, viz.:

A. 1 part 40 % formaldehyde in 70 parts of gypsum
B. 1 „ „ 35 „

The variety of wheat was Iron III and the seed was artificially infected with bunt spores from other crops of different varieties of wheat.

Results:

Control plot	27 %	bunted ears
Plot from seed treated with strength A	1 %	„
„ „ B	1·7 %	„

The seed was treated on March 27th and drilled 2 days later.

Second year's experiments—1925.

This year, the same variety, Iron III, was used and was sown in late autumn of 1924 in order to see whether it was possible to get a higher percentage of bunted ears in the control plot than in the previous year. Unfortunately the birds took most of the seed and the plots were a failure.

It was decided to sow again in the Spring, and in addition to the variety Iron III, duplicate plots with a Spring wheat, Red Marvel, were also sown.

In order to see whether the gypsum alone, without the formaldehyde, exercised any control on this disease, a plot was included in which the seed was treated with gypsum powder only.

The variety Iron III was treated on March 5th and sown on March 6th, and Red Marvel was treated on April 23rd and sown on April 24th.

All the seed was again artificially infected with bunt spores from other crops of different varieties.

Results:

Iron III.

Control plot	24 %	bunted ears
Plot from seed treated with gypsum only	11 %	,,
,, ,, powder Strength A ...	6 %	,,
,, ,, powder Strength B ...	1·2 %	,,

Red Marvel.

Control plot	15 %	bunted ears
Plot from seed treated with gypsum only	7·2 %	,,
,, ,, powder Strength A ...	5·2 %	,,
,, ,, powder Strength B ...	2 %	,,

Third year's experiments—1926.

Two varieties of wheat were again used, viz. Martin and Little Joss.

Only three plots in each variety were sown, (*a*) the control plot (not dressed), (*b*) seed dressed with powder strength B (1 part 40 per cent. formaldehyde in 35 parts of gypsum), and (*c*) seed dressed with gypsum powder only.

It is important to note that in these experiments the seed wheat was *naturally infected* to begin with and heavier infection was made by using bunt spores from the same sample of seed. This was because the natural infection was only slight.

The seed was dressed on October 31st, 1925, and sown on November 24th, 1925.

Results:

	Bunted ears	
	Martin %	Little Joss %
Control plot	85	66
Seed treated with powder Strength B	75	31
,, gypsum powder only	88	62

Fourth year's experiments—1927.

As the control exercised by the formalin-gypsum powder, strength B, was very poor in 1926, it was decided to use gypsum powders incorporating more formaldehyde. The following strengths were made up:

B. 1 part 40 % formaldehyde in 35 parts gypsum (by weight)
C. 1 ,, ,, 20 ,, ,,
D. 1 ,, ,, 10 ,, ,,

Stronger mixtures than D could not be used as they were too damp for practical purposes and did not adhere to the wheat grains.

Two varieties, Martin and Little Joss, were again used. They were naturally infected with bunt spores from the same crop, as was done in 1926.

The seed was dressed on November 12th, 1926, and sown on November 23rd, 1926.

Further germination tests in the laboratory showed no damage to the germination capacity of the dressed seed, even after treatment with the strongest powder D; but in the field later there was a much thinner plant on the plot treated with strength D, so that some damage must have been done to the seed by the heavy formaldehyde content of the powder, although this did not show in laboratory tests.

Results:

	Bunted ears			
Variety	Control %	Strength D %	Strength C %	Strength B %
Little Joss	70	10	40	70
Martin	90	68	80	86

Discussion of results.

The control of Bunt exercised by the formalin-gypsum powder in the first two years was sufficiently good to warrant a further trial. In the third year, however, this powder showed no control of the disease in the variety Martin and very little control in Little Joss. In the fourth year also there was practically no control of Bunt in Martin and only a moderate control in Little Joss even when the strongest mixture was used.

It is difficult to account for the different results obtained in the third and fourth years. The chief factor different was that infection was made by bunt spores obtained from the same sample of wheat: in other words, infection was *natural*. This might be the cause of the much greater percentage of bunted ears obtained in the control plots.

On the other hand, the wheat used in the first two years was a *clean* sample and infection of the seed had to be made artificially with bunt spores obtained from other crops of different varieties. In both these years the percentage of bunted ears in the control plots was much lower, although the rate of infection was the same as in the third and fourth years. There seems to be some evidence here to support the view that *Tilletia tritici* is a fungus comprising a number of biologic forms.

Summary.

A series of experiments over a period of four years is described in which attempts are made to control Bunt in wheat by dressing heavily infected seed with a dry powder or dust composed of formaldehyde and calcined gypsum.

Good control was obtained in the first two years when clean seed, *artificially* infected with bunt spores obtained from crops of different varieties, was used.

In the last two years the seed was heavily infected *naturally* by bunt spores obtained from the same crop. Under these conditions, the formalin-gypsum powder exercised little or no control of Bunt.

The experiments seem to provide some evidence to support the view that *Tilletia tritici* is a fungus comprising a number of biologic forms.

While going to press, attention has been drawn to a paper by J. D. Sayre and R. C. Thomas, which appeared in the October, 1927, number of *Science* (Vol. LXVI, No. 1713). In this paper the authors give the results of one year's experiments on the control of oat smut using a dry powder incorporating formaldehyde with charcoal or infusorial earth.

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THE USE OF TETRACHLORETHANE FOR COMMERCIAL GLASSHOUSE FUMIGATION

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Introduction.

The use of tetrachlorethane as a specific for White Fly (*Trialeurodes* (*Aleurodes*) *vaporariorum* West.) appears to have been first studied in this country by the late Prof. Lefroy and G. Fox Wilson in 1915, but their work has not been published. In trying out various chemicals in order to discover a reasonably cheap and non-poisonous specific they found that this material used at one-tenth of the molecular weight in grams per 10 cu. ft. gave a complete kill of adults and nymphs on *Barleria cristata* heavily infested with the pest, the eggs being unaffected.

In 1920 and 1922 Lloyd(1, 2) investigated its use on tomatoes and cucumbers and considered it a good fumigant for White Fly owing to its simplicity in application, but on account of the high cost it could not compete with the older method of cyaniding.

Speyer(3) in 1925 examined the effect of tetrachlorethane vapour on chrysanthemums and found nearly all varieties were affected by the vapours of the fumigant to a greater or lesser extent.

In 1926 G. Fox Wilson(4) published an account of its use as a glasshouse fumigant and gave details concerning method of application, conditions and concentration, and a list of plants safe and otherwise as a guide upon which the fumigant could be used.

The writer(5) in 1927 published a note under the title of this paper which is elaborated herein.

Lloyd(6) in commenting upon certain statements made by the writer offered suggestions which are referred to later under "Discussion of Results."

Tetrachlorethane was largely used as an aeroplane dope during the war, and it has since had an extended industrial use as an organic solvent, in consequence of which manufacturing processes have been improved that have considerably cheapened its cost.

It was first introduced into commercial horticultural practice by the writer in 1920, and is now extensively used by both the professional and the amateur.

Thanks are due to Mr G. Fox Wilson for kindly reading through the MSS. and for valuable criticisms.

Object of Investigation.

This paper is a record of work carried out from 1920 to 1926 to ascertain the best method of application under commercial conditions, and the lowest concentration which will effectively control certain pests, on a variety of glasshouse plants.

Outline of Scheme of Work.

Commercial tetrachlorethane is a volatile fluid at ordinary temperature, having a specific gravity of 1·6, and a boiling point of 147° C. Like chloroform its volatility increases with rise of temperature. The following methods of application were used:

1. Sprinkling the fumigant along the paths of the house.
2. Pouring on to heaps of coke contained in seed boxes placed at intervals along the paths.
3. Vaporising by means of lamps.
4. Impregnating sacks hung at intervals from the wires of the house.
5. Atomising by means of a mist "sprayer."

Preliminary fumigations.

Fumigations were carried out in a house of 1000 cu. ft. capacity equipped with well-fitting doors and vents containing a variety of greenhouse plants in pots.

Leaves (tomato) badly infested with White Fly in egg, nymphal and adult stages were introduced in circular cages covered with fine muslin. These were hung at intervals from the ridge board. A series of fumigations were carried out in the absence of the plants to test the effects of various concentrations of the fumigant upon the fly (see Table I). The method of application in these preliminaries was to sprinkle the fumigant on to sacks hung from the wires.

Method of recording.

After each fumigation the cages were removed and kept in an airy position for at least 48 hours, then opened and examined, the fly being carefully shaken from the cage on to black paper. The leaves were also

removed and shaken to remove any adhering adults. The kill in the case of the fly stage was determined by counting the number which showed no signs of recovery after 48 hours.

With the nymphal stage the leaves were kept in water (changed daily) in a warm and light position for 14 days, and again examined for appearance of any newly-hatched adults. No counts were made in the case of the eggs.

Table I.

Conc. per 1000 cu. ft.	Temp. ° F.	Humidity %	Adults		Scale	
			Alive %	M. and D. %	Hatched %	M. and D. %
1½ fluid oz.	60–65	80	28	72	100	—
2½ „	65–70	80–90	20	80	100	—
5 „	65–70	80–90	5	95	100	—
10 „	70–72	80–90	0	100	5–10	90–95
15 „	60–70	—	0	100	2	98
20 „	65–70	—	0	100	—	100

M. = moribund and not likely to recover. D. = dead.

A third series of fumigations was carried out to determine if tetrachlorethane had any effect upon certain other pests.

The method of fumigation was precisely the same as before. This time leaves infested with Red Spider (*Tetranychus telarius*), Green and Black Fly (*Aphides* spp.) and Mealy Bug (*Dactylopus longispinus*) were introduced into the cages and examined. No counts, however, were made, the kills being estimated (see Table II).

Table II.

Conc. per 1000 cu. ft.	Red Spider		*Aphides* G. and B.		Mealy Bug	
	Active %	M. and D. %	Active %	M. and D. %	Active %	M. and D. %
2½ fluid oz.	100	0	100	0	100	0
5 „	100	0	100	0	100	0
10 „	100	0	100	0	90–95	5–10
20 „	100	0	80–90	10–20	95	5

COMMERCIAL FUMIGATIONS.

These were conducted in large commercial tomato glasshouses in various parts of the country, where the different methods of application were tried under similar conditions as far as commercially possible. No counts were made but the kill estimated as follows.

If after 2 days following the fumigation the adults were still motionless on the ground and no "fly" rose from the plants when

Table III.

Effect upon plants.

Period of fumigation 12 hrs.
Concentration 5 fluid oz. per 1000 cu. fit. Temp. 70° F.
Condition of atmosphere in house damp.

Plant	Unaffected	Slightly affected	Badly affected
Arum	+	−	−
Adiantum elegans	+	−	−
Antirrhinum	+	−	−
Asparagus plumosus	+	−	−
Begonia	+	−	−
Canterbury Bell	+	−	−
Carnation	+	−	−
Coleus	+	−	−
Cucumber	+	−	−
Caladium	+	−	−
Cyclamen	+	−	−
Deutzia	+	−	−
Fuchsia	−	+	−
Freesia	+	−	−
French Bean	+	−	−
Geranium	+	−	−
Gloxinia	+	−	−
Grape	+	−	−
Grevillea robusta	+	−	−
Heliotrope	+	−	−
White Hydrangea	+	−	−
Kochia	+	−	−
Maidenhair Fern	+	−	−
Marrow	+	−	−
Mimulus (Annual)	+	−	−
Nephrolepsis	+	−	−
Petunia	+	−	−
Polypodium glaucum	+	−	−
Primula malacoides	+	−	−
Sobralia	+	−	−
Marguerite	+	−	−
Pteris albo lineata	+	−	−
Pteris Wimsettii	+	−	−
Rose in Bloom	+	−	−
Tomato	+	−	−
Tradescantia	+	−	−
Lilium longiflorum	+	−	−
Salvia	−	+	−
Humea elegans	−	+	−
Pelargonium (Cape)	−	+	−
Camellia	−	+	−
Asparagus Sprengerii	−	+	−
Dahlia	−	+	−
Aspidistra	−	+	−
Azalea	−	+	−
Balsam	−	−	+
Canna	−	−	+
Calceolaria	−	+	−
Cineraria	−	−	+
Chrysanthemum	−	−	+
Crassula	−	−	+
Lemon Plant	−	−	+
Sweet Pea	−	−	+
Pink Hydrangea	−	−	+
Acer pseudoplatanus	−	+	−

violently agitated a 100 per cent. kill was recorded, where a few "fly" appeared 90 per cent. was recorded. Houses heavily infested with the pest were selected for these trials.

Approximately 150 fumigations were carried out.

Table IV.

Method of application (see p. 252)	Period of fumigation hrs.	Conc. per 1000 cu. ft.	Temp. ° F.	Humidity %	% M. and D. of adults	% M. and D. of scale
No. 1	12	2½ fluid oz.	60–70	80–90	Less than 90	Not determined
		5 „	„	„	90	„
„ 2	12	2½ „	„	„	Less than 90	„
		5 „	„	„	„	„
„ 3	12	Caused some decomposition of fumigant and corrosion of the aluminium pan, no further fumigations carried out by this method				
„ 4	12	2½ fluid oz.	60–70	80–90	90	Not determined
		5 „	„	„	100	„
„ 5	12	Discontinued as the spray fell upon the foliage causing severe scorching				

All fumigations were carried out at night; time starting at about 6 p.m.

Discussion of Results.

In some districts it is the practice to "bed in" chrysanthemums after clearing a crop of tomatoes, dispensing with a central path and replacing with two side paths.

Where Method of Application No. 1 was used it was found that chrysanthemums subsequently planted gradually died off as though affected by a root rot. It was noticed that the area affected corresponded with the position of the path that had been sprinkled with tetrachlorethane.

The plants were removed and examined, but no sign of disease could be detected.

The soil was examined and smelt strongly of the fumigant 6 months after, and it appeared to be gradually permeating the soil to a distance of several feet on either side of the path.

The affected soil also showed a remarkable freedom from the usual soil insects found in a tomato house.

Some small scale laboratory experiments were conducted to ascertain the toxicity of the fumigant in soil against wire-worm and wood-lice, which proved to be very promising, but in view of the length of time

the chemical remained in the soil and the experience gained above it was decided to proceed no further on these lines.

The selective toxic action of tetrachlorethane to insects is interesting and requires some explanation. Lloyd(6) recently suggested that being an organic solvent it has some action upon the waxy covering of the "fly" in both the scale and adult stages causing it to run, thereby upsetting the delicate mechanism of breathing.

He considers that it is probably not a tissue poison in the proportions used since, if it were, it is difficult to imagine Green Fly escaping its action.

These views gain support from the fact that Mealy Bugs are affected by the fumigant.

This may also explain the varietal susceptibility of certain plants to the action of the fumigant, because it is difficult to understand why a sappy and tender-growthed plant like a cucumber will withstand high concentrations whilst hard-wooded plants such as chrysanthemums are affected by very small quantities.

The results from using the different methods of application indicate that the most satisfactory fumigations are obtained by impregnating sacks, hung from the wires, with the fumigant.

A commercial control of the adult fly can be obtained by using $2\frac{1}{2}$ to 5 fluid oz. per 1000 cu. ft. providing the temperature is maintained at 65–70° F., and the house is reasonably tight and the fumigation proceeds for 12 hours.

High concentrations must be used to destroy the nymphal stage, but it is considered that three fumigations at 5 fluid oz. concentration, given at intervals of a week or 10 days, should be sufficient to keep in check an ordinary infestation.

Some growers fumigate once a fortnight as a precautionary measure, using 2–$2\frac{1}{2}$ fluid oz. in the early part of the season.

Care must be exercised when using this material, owing to the possibility of decomposition during storage with the formation of free hydrochloric acid.

The product should be tested before use if held in stock for any considerable length of time.

The list of plants given in Table III showing susceptibility or otherwise to tetrachlorethane must only be taken as an indication or guide.

SUMMARY.

1. Various methods for the application of tetrachlorethane as a fumigant for the control of White Fly under commercial horticultural conditions are discussed.

2. The lowest concentration that appears to give a control has been suggested.

3. The fumigant appears to be selective in its action upon White Fly and Mealy Bug. Certain species of Aphides seem to be unaffected.

4. Certain plants show a varietal susceptibility to the action of the fumigant.

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(*Received November 17th*, 1927.)

A GARDEN CHAFER ATTACK

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(With Plate XIV and 2 Text-figures.)

The Garden Chafer (*Phyllopertha horticola* L.), despite its name, does more harm on farms than in gardens, for, whereas the beetles live for 2 or 3 weeks only and feed on flowers and bracken, the larvae, inhabiting grassland, live for 8 months or more and not infrequently cause, when numerous, much loss of crop. The following account of an attack witnessed in 1919–20 confirms the recommendation given by Curtis, Warburton and others that the best time to adopt control measures is in June when the beetle is swarming upon its food plants.

The farm, known as Laund House, where the observations were made, is situated on the side of Earl Seat in Upper Wharfedale, about 2 miles above Bolton Abbey, the terrace on which it lies being about 150 ft. above the river and about 500 ft. above sea-level. As is usual in the district, the farm is mostly composed of grassland of which small enclosed portions serving as meadows are mown in July or August and grazed in winter and spring. In 1919 a complaint was received that for several years the hay crop had been getting lighter and poorer in quality; and in November, when the writers saw the meadows, the turf, especially of one, a five-acre field, was found in many places to be in such a bad condition and so loosely attached that the cattle were either unwilling or unable to graze it.

When the affected areas were examined more closely numbers of a well-grown, pale-coloured grub were found in the soil, their presence amply confirming the opinion expressed by the tenant, that the damage to the grass was due to "white grub," specimens of which he had repeatedly found in the soil at a depth of from 1 to 6 in. Although locally regarded as the larva of the Cockchafer, the grub proved to be that of the allied Garden Chafer, which differs, among other things, from the

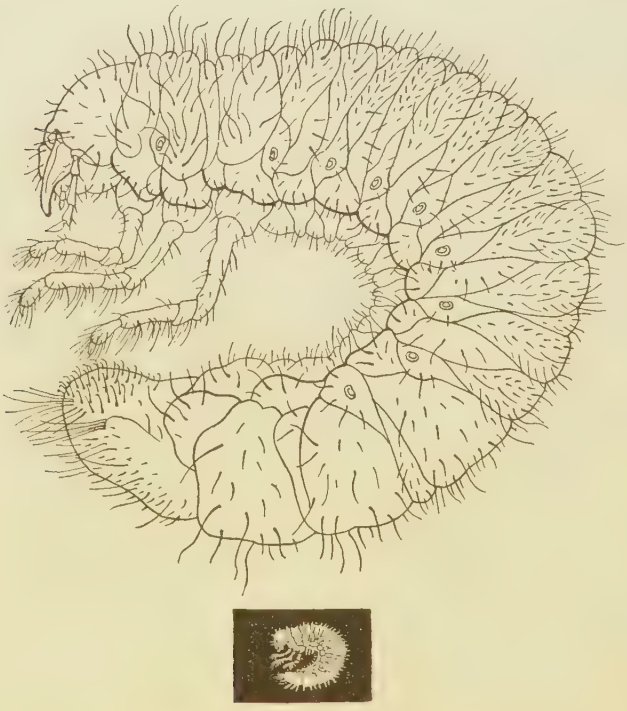
Cockchafer in being smaller in size and in completing its life-history in a year.

It was found that the grub, feeding exclusively on roots and other fibres in the soil, had to a marked degree severed the attachment of the herbage to the ground. In many places, indeed, so completely had the grub destroyed the roots that the sward, while still retaining its vitality, lay loose on the surface and could be pulled up in handfuls. It was noticed, too, that, when treading the affected sward, one's foot, instead of experiencing the firm, springy resistance characteristic of healthy well-grazed turf, sank in almost as deeply as into marshy ground. In these areas the grass had become much discoloured and, being uncropped by the cattle, had grown unduly long and coarse. Starlings and other birds had flocked to the fields and, although instrumental no doubt in destroying the grub to some extent, had increased the general unsightliness by scattering loose fragments of herbage about the surface.

In the following spring (1920) the larval attack waned, whereupon the turf developed new roots and began to recover; and it seems probable that if the meadows had originally been subjected merely to one season's attack, they would not have suffered any lasting harm. As the matter stood, however, the attack had been repeated each season for a period of 10 or 12 years, the effect being, in the tenant's opinion, gradually to remove the small fine-leaved kinds of grass, thereby rendering the sward coarser and of inferior quality.

The larvae pupated during May at a depth of about 4 in., the pupae being often found enveloped in a loose shroud-like membrane looking at first sight like a cocoon but which proved to be the cast-off larval skin, longitudinally fissured but otherwise intact. On June 8th the beetles were seen about the lawn and the flower-beds of the garden, and during the following 2 or 3 weeks became very numerous, flying to some degree in the wood but chiefly in the meadows where they had passed their larval stage. Indeed, these upland meadows, with their varied herbage and close screen of woodland, seem to have afforded ideal conditions for the beetle's development. Moreover, the soil, being derived from Millstone Grit, is sufficiently sandy and open in texture for the needs of the larva, which is more active in its movements than its rather clumsy form would suggest (see Fig. 1). Very few of the chafers, it may be added, were seen beyond the immediate vicinity of the farm, but at Ilkley, lower down the valley, they were noticed by the late Prof. L. C. Miall, who reported them to be fairly numerous.

Entirely neglecting the grass, the beetles when feeding selected broad-leaved plants, notably buttercup, *Rumex* and clover, the flowers of which they often completely destroyed. Still more relished apparently were the young fronds of bracken which they found growing alongside the boundary walls. Plentiful everywhere in the surrounding woods, bracken fern has here and there pushed its underground shoots through



Natural size

Fig. 1. *Phyllopertha horticola* L. Larva. × 7.

the rough-hewn uncemented walls, and the fronds arising therefrom have furnished the chafers not only with a much appreciated supply of food but also, it should be added, with their favourite resort when mating.

The chafer's liking for bracken and its habit of feeding when the fronds are unfolding—which habit has perhaps suggested its name of "bracken *clock*"—could no doubt be made use of when its distribution is being studied. There would, however, be some risk of confusing

chafer-caused injuries with wounds made by other bracken-feeding insects and especially with the discoloration caused by late frosts, the scorching effects of which are, when the fronds are seen from a distance, not unlike the marks made by these beetles (see Pl. XIV).

The beetles, although somewhat inactive, occasionally fly about the fields in sunny weather, and since their flight is steady and not too rapid they are, when flying, easy to capture. When resting on bracken they can be taken with even less trouble, for, if disturbed, instead of flying away they commonly conceal themselves by falling into the undergrowth below. There are, indeed, in the life of the Garden Chafer and its association with bracken, several features which combine to make it possible to capture the beetles wholesale by unusually simple means, viz. the short duration of the adult stage (flying stage); the beetle's comparative inertness, and its indifference to the presence of anyone standing near; its habit of dropping when disturbed, and the ease with which the beetles can be caught when falling from a convenient height, *e.g.* from bracken fronds; and, lastly, its habit, when swarming, of restricting itself to some small selected spot such as, for instance, an isolated clump of bracken.

In June, the much recommended plan was tried of taking the beetles when they were resting on the bracken. Collecting them by hand proved too slow, and sweeping them into a net had the disadvantage that they clung to the muslin and required to be picked off one by one, a troublesome task since they possess unusually strong claws. Paper, cloth, tiles, etc. smeared with tanglefoot and placed on the ground and walls beside the bracken, required no further attention and were fairly successful; but the best method was to shake them off the bracken on to a wide-lipped scoop and slide them into a box attached to the other end, care having been taken to attach the box in such a way that when the beetles fell in they could not crawl out again (see Fig. 2).

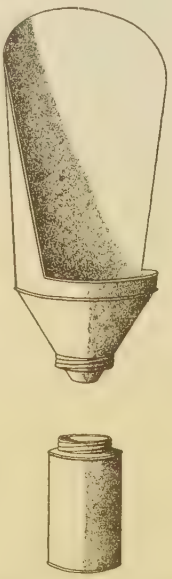


Fig. 2. Garden Chafer trap. When in use, the two parts are screwed together.

By these means the beetles were destroyed in large numbers, with the result, no doubt, that very little egg-laying took place that year.

No complaint of an attack was received during the following winter (1920–21), and in November 1921, when the meadows were again visited, the turf was found to be firmly rooted and carrying a healthy-looking sward. Visits were made in June 1924 and 1925, August 1926 and July 1927; but very little trace and finally none at all of either the beetle or its damage could be discovered, and although it is difficult in field work of this kind to assign effects to their causes there seems no good reason to doubt that this improvement was due to the control measures which were taken in June 1920.

Summary.

1. The good effects obtained by capturing the beetles when they are swarming on bracken last apparently for several years.

2. A form of trap is described, suitable for capturing them when they are resting on the fronds.

The writers are indebted to Miss E. M. Wright for the drawings of the larva and injured bracken.

(*Received January* 14*th*, 1928.)

Upper surface

Under surface

Pinnule of Bracken eaten by the Bracken-Clock

Pinnule of Bracken damaged by Frost

E. M. Wright
23. *vi.* 20

TAYLOR & THOMPSON.—A Garden Chafer Attack (pp. 258–262).

THE BIONOMICS OF *APION ULICIS* FÖRST. (GORSE WEEVIL), WITH SPECIAL REFERENCE TO ITS RÔLE IN THE CONTROL OF *ULEX EUROPAEUS* IN NEW ZEALAND[1]

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(With Plates XV–XVII and 3 Text-figures.)

CONTENTS.

1. Introduction.

Biological control has, during recent years, made considerable advance in its position in economic entomology. This has resulted chiefly from the study and application of biological control of insect pests. Latterly, however, a further aspect of this subject has been considered, namely, the biological control of noxious weeds; it is with this section that the present paper is concerned.

The works of Perkins and Swezey(15), Koebele(14), Alexander(1), Imms(12) and Tillyard(19) have dealt exhaustively with the present position of this subject, so that a brief summary will here suffice.

It is obvious that the introduction of insects into a country as a means of controlling a noxious plant, can only be adopted when the

[1] Part of thesis approved for the Degree of Doctor of Philosophy in the University of London.

nature of the problem is sufficiently grave as to necessitate an attempt being made with every possible safeguard, and under most critical scientific supervision.

The pioneer work in the control of noxious weeds was done in the Hawaiian Islands, where the colonisation of a number of species of insects introduced from Mexico afforded an appreciable control of the injurious plant *Lantana camara*. Investigations regarding the repression of nut-grass (*Cyperus rotundatus*) by the introduction of insect enemies from the Philippines, has also been the subject of experiment in the Hawaiian Islands.

The most formidable attempt at the control of noxious weeds by means of insects is that of the Prickly Pear campaign in Queensland and New South Wales. So serious became the spread of the introduced plant, that in 1919 a "Commonwealth Prickly Pear Board" was established to deal with the problem. Insect enemies of the Prickly Pear were searched for in the warmer parts of America, and the first consignment shipped to Australia in March 1921. The outcome of this, and later, importations is that Cochineal insects of the genus *Dactylopius* have already destroyed large areas of this noxious weed. Recent reports from the areas where this work of weed control is being carried out are extremely encouraging.

A great stimulus to this new line of work has been provided by the scheme that has been financed by the Empire Marketing Board in conjunction with the New Zealand Government and the Cawthron Institute, Nelson, to ascertain the possibilities of establishing a control of noxious weeds in New Zealand by the introduction into that country of appropriate insects. A proportion of the above grant has been allocated by the Cawthron Institute to the Rothamsted Experimental Station, and it is with the financial assistance thus available that the experiments involved in the present paper have been carried out. The work has been prosecuted under the direction of Dr A. D. Imms whose valuable advice is gratefully acknowledged. The writer is also greatly indebted to Dr R. J. Tillyard who instigated the work in New Zealand.

Ulex europaeus L. (common gorse or furze) is included among the plants scheduled by the New Zealand Government (21) as noxious weeds. According to Thomson (18) it must have been introduced at an early date, as it is noted that Darwin observed plants of gorse in 1835. Since its introduction it has spread very rapidly and now covers large areas of ground, threatening to render derelict some of the most valuable pasture land of that country. A similar occurrence is in the Hawaiian

Islands, where it is stated that 17 years ago the first and single plant of *Ulex europaeus* was observed. Subsequently this plant has spread at an extraordinary rate, and the possibilities of devastation of valuable land has been so keenly felt that it has been proposed that large sums of money be spent to attempt to eradicate this weed. The methods used are chiefly mechanical, gangs of men being employed to dig out and burn the young seedlings. That the plant spreads by means of its seed has been fully proved, owing to the fact that young seedlings are found many miles away from the original plant.

In the young stage *Ulex europaeus* is used for sheep grazing and, further, being a leguminous plant it is highly beneficial in unmanured areas. The problem then is one of *control* rather than *eradication*. An insect which is effective in destroying the seeds will considerably assist in solving the problem. As will be seen later, a survey of the damage caused by *Apion ulicis* to gorse seeds in Great Britain has been made, and it is evident that this insect should receive full consideration.

2. Synonymy of *Apion ulicis* Först.

Apion ulicis Först. is the name under which it is most commonly known; it was originally described by Förster(6). It was described by Fabricius(5) as *A. nigrirostre* and again in 1808 by Kirkby as *A. ilicis*. Gyllenhal(10) named it *A. carpini*, while in 1882 Gredler described it under the name of *A. sarothamni*. Schilsky(17) considered it a variety, *nigripes*.

3. Description of Adult (Fig. 1).

The generic characters, viz. pear-shaped body with long, slender curved rostrum, together with long trochanters and straight antennae, are well marked. The *body* is convex, with integument rugosely punctured and pitchy black in colour, but with the exception of the eyes, rostrum, antennae, scutellum and joints of the legs, it is densely squamose, the thick white scales giving it a characteristic grey colour. *Head*, short, with the black convex eyes moderately separated; the black rostrum, shorter in the male than in the female, is narrow and slightly curved. The *antennae*, sparsely covered with fine white hairs, are straight, slender, clubbed, and arise from the base of the rostrum, the point of insertion being marked by a strong black chitinous projection. The *thorax*, practically equal in length and breadth, is slightly narrower in front, rounded behind the middle and contracted at the base; the scales are more or less irregularly scattered but are wanting from a longitudinal area in front of the scutellum. The *scutellum* is smooth and black. The

elytra are convex, and have the scales arranged in the form of longitudinal striae, the second of which is united at the apex to the eighth. The *legs* are black, anterior pair are sometimes reddish, and except for the joints, they are covered with scales; they are comparatively long with well-developed coxae; femora slightly dilated at the apex; tibiae more or less straight with long tarsi terminating in a bifid claw. The *abdomen* is covered with scales ventrally, these being absent at the junction of the segments. Size 2–2·75 mm.

Sexual Differentiation.

Generally speaking, the male is slightly smaller than the female, but the sexes are easily distinguished by the length of the rostrum, the proportions of that of the male to that of the female being 7 : 12. The antennae are proportionately shorter in the male. No apparent difference in the abdominal segments occurs.

Description of Mouth Parts of the Adult.

Dorsal view (Fig. 2 *a*). The mouth parts of the male are similar to those of the female; those of the female are described. The elongate nature of the rostrum results in a modification of the normal mouth parts. The *labrum* and *clypeus* are not present and the *epistoma*[1] (*epi*) is merely indicated by a faint line which divides off the apex of the rostrum. The *mandibles* (Fig. 2 *b*) are well developed and tri-dentate in form, the apical tooth is the largest, and the lateral one curves slightly dorsally. In structure the mandible and its attachments closely resemble that of *Pissodes strobi*, as figured by Hopkins (11). The ventral articulation has a median "ball" condyle (*c*) surrounded by a deep fossa—the *ginglymus* (*gm*). The abductor (*ab m*) and adductor (*ad m*) muscles are attached to the sides of the fossa. The so-named *pharyngeal bracon* (*ph b*) of Hopkins is present and has its surface covered with papillae. This structure extends into the pharynx, and the fact that the papillae point posteriorly suggest that the organ functions along with the ligula and lacinia in facilitating the passage of the food within the elongate rostrum.

Ventral view (Fig. 3). The ventral side of the rostrum is entirely complete, there being no hypostomal punctures of any kind on this surface. The *maxillae* (*mx*) are well developed except that the *cardo* (*cd*), *subgalea* (*sg*) and *stipes* (*st*) are ill-defined, being represented by one broad lobe without sutures. The *palpifer* (*f*) is large and bears a stout 2-jointed *maxillary palpus* (*mx p*), the apex of which is fringed with

[1] The terminology here used is that of Hopkins (11).

tubercles and the base of the terminal segment possesses strong spines. The *lacinia* (*lc*) is also well developed and covered with papillae. The *labrum* consists of an elongate *submentum* (*sm*) with its apex more or less rounded supporting the *mentum* (*mt*) which is as long as the submentum. The mentum has two strong elongate spines slightly posterior to its middle line and anteriorly bears two unsegmented *labial palpi* (*l p*), which also possess strong hairs. There is a well-developed undivided *ligula* (*lg*) the surface of which is covered with dense papillae.

Reproductive Organs.

No detailed study of development has been undertaken, but reproductive organs of both sexes have been examined periodically from the time of emergence from the pod in the Autumn to the period of mating in the Spring. When the weevils emerge from the pod the reproductive organs are exceedingly immature, the ovaries being merely fine tubules. It is interesting to note further, that if for some reason or other, the pod does not open until Spring—one instance in May 1927 was noticed—the reproductive organs of these imprisoned weevils still remain immature and do not begin development until after the weevil has escaped and commenced feeding. This probably accounts for the fact that oviposition is spread over a comparatively long period. Normally the reproductive organs are mature about February or March.

Male Reproductive Organs.

The male reproductive organs are shown in Fig. 4.

The *testes* (*t*) are bifollicular, each follicle being globular and of equal size, white in colour and, when mature, measures 0·23 mm. in diameter. The paired *vasa deferentia* (*v d*) are comparatively short and immediately after leaving the testes become slightly swollen, this region probably being that of the *vesicula seminalis* (*v s*). Well developed *accessory glands* (*a g*) measuring 0·8 mm. in length are present; these also arise early along the course of the *vasa deferentia* and are swollen at their apices. The *ejaculatory duct* (*e d*) follows from the junction of the paired *vasa deferentia* and leads into the *transfer apparatus* (*tr*) where it is surrounded by the strongly chitinous walls of the latter. The main section of the chitinous apparatus gives rise anteriorly to two chitinous rods 0·6 mm. long, arranged in a U-shape. The larger portion mentioned is 0·8 mm. long and 0·08 mm. broad at the point where the arms join it; it is slightly curved and terminates in a fine point surrounding the *aedaegus* (*a*). There is also an additional, more or less straight, chitinous rod (*r*)

measuring 0·4 mm. in length, which serves as a further support of the transfer apparatus. Strong longitudinal muscles (*m*) are attached to the proximal end of this rod and also to the proximal and distal end of the entire transfer apparatus. These muscles serve in extending the apparatus during copulation. There is no indication of claspers.

Female Reproductive Organs.

The female reproductive organs are shown in Fig. 5 and measure from the vaginal opening to the terminal filament from 2·0–2·5 mm.

The *terminal filaments* (*t*) are exceedingly slender and very easily separated. There are four *ovarioles* (*o*) measuring 1–1·5 mm. in length and 0·1–0·12 mm. in maximum breadth. The presence of eggs in the *vitellarium* (*vit*) is easily detected in mature specimens. The ovarioles unite to form the *oviducts* (*od*) which are short, measuring 0·05–0·8 mm. These lead into the common duct or *uterus* which measures 0·5–0·7 mm. in length and at its anterior end is 0·12 mm. in breadth. The uterus terminates in the *vagina* (*vg*) which is slightly wider and is protected posteriorly by chitinous sclerites. The vagina gives rise dorsally to a pouch-like *bursa copulatrix* (*b c*) of 0·42 mm. length and 0·15 mm. breadth; it is slightly curved at its distal end. The *spermatheca* (*sp*) is strongly chitinised and curved in form; it unites with the uterus by means of a fine *spermatic duct* (*sp d*). There is a small spherical *accessory gland* adjacent to the spermatheca. The long chitinous rod (*r*) of the ovipositor measures 0·8 mm. in length, it is swollen at its apex, and strong longitudinal muscles (*m*) are here attached. Two shorter chitinous spicules are present at its base. There is no indication of an egg calyx commonly found in Rhyncophora.

4. The Egg (see Fig. 13).

The egg is smooth with delicate yellow chorion. At the time of oviposition it is elongate in shape measuring 0·4 × 0·2 mm., later it assumes a glossy white appearance and becomes more round in shape measuring 0·35 × 0·25 mm.

5. The Larva (Fig. 6).

The larva is typical of the Curculionidae being eruciform and apodous. It is a yellowish white, fleshy grub, and on emergence from the egg measures 0·5–0·6 mm. long by 0·25 mm. in width. At maturity the larva is very plump and practically incapable of movement; its measurements are as follows: length of body, including head capsule 2·5 mm., breadth

in abdominal region 1·3 mm., head capsule 0·15 mm. long and 0·16 mm. broad just behind the middle line. The entire body is strongly crescentic and sparsely covered with fine hairs.

Head.

Dorsal view (Fig. 7). The head is well developed, testaceous in colour in the early stages but becoming darker as it reaches its final instar. It has a few scattered hairs on its surface, the normal arrangement of which is shown in the figure.

The entire head, excluding the mandibles is as broad as long; the *epicranial plates* (*ep l*) are large and are rounded laterally. The *epicranial suture* (*e s*) is very well marked, there being a gap between the epicranial plates at the base of the head; the lateral arms (*l es*) of this suture distinctly separate the plates from the frons. The *frons* (*fr*) is triangular in form and anteriorly there is a slight indication of an *epistoma* (*ep*)[1]. There is no indication of eyes or ocular pigment. The *antennae* (*a*) are present as stout papillae with two small tubercles at their bases. The *mandibles* (*mn*) are strongly chitinous, stout and triangular in outline. They are tridentate, the apical and subapical teeth being more acute in form than the smaller lower tooth, which is sometimes merely a prominence. There are four well-developed spines on each mandible arranged normally as figured. The *clypeus* (*cl*) is quite distinct and is longer laterally than in the median line; it is devoid of spines. The dome-shaped *labrum* (*e*) is well covered with spines especially at the apex, the arrangement as figured is normal and characteristic.

Ventral view (Fig. 8). The *maxillae* (*A*) are well developed and, with the exception of the galea and subgalea, all the sclerites are distinct. The *cardo* (*cd*) is stout and club-shaped, and unites with the larger stipes (*st*), the latter having several strong spines as indicated on the figure. The *palpifer* (*f*) is short, slightly broader than long and bears the *maxillary palpus* (*mx p*) which is represented as a stout elongate unsegmented lobe, fringed with papillae at its apex. The *lacinia* (*lc*), fused as it is with the galea (*gl*) and *subgalea* (*s gl*) is in the form of an elongate lobe, the interno-lateral face of which is fringed with lacinial teeth. The *labium* (*B*) is large and slightly broader than long, the broadest line being nearer the base. The *submentum* (*sm*) comprises most of the labium and is rounded laterally; there are a few strong spines on its surface as indicated. The *mentum* (*mt*) is triangular, the apex of which reaches beyond the middle of the head, this sclerite also has scattered

[1] See previous footnote.

spines on its surface. Anteriorly there is a faint suture indicating the division of the mentum and *prementum* (*pm*): the latter sclerite is very narrow and has a ridged free margin. The *labial palpi* (*l p*) are short stumpy unsegmented lobes with their apices fringed with papillae.

Thorax.

The three sclerites of the thorax are clearly defined, the prothorax being slightly reduced. The *prescutum* (*psc*), the *scutum* (*sc*) and the *scutellum* (*scl*) are only feebly indicated. The *pleurites* (*pl*) as a whole are well defined in the thorax of mature larvae but the individual constituents are not indicated.

There is a biforous spiracle at the junction between the prothorax and mesothorax; this will be described later.

Abdomen.

The abdomen possesses 10 distinct segments, the sutures becoming less distinct anally: in each of the segments of the notum the elements are indistinct. The 10th segment is considerably reduced and serves occasionally as an organ of locomotion. The *pleural groove* (*pl g*) is well marked and there are indications of the *hypopleural* fold (*hlp*) and the *sternellar* fold (*st*). Hairs are scattered over both abdomen and thorax.

Spiracles (Fig. 9).

There are eight pairs of spiracles. The first pair situated between the prothorax and mesothorax are *biforous* in form (Fig. 9 A). Each consists of the annular sclerite or *peritreme* (*pr*) which surrounds it, the spiracular opening (*o*) which leads into the *atrium* (*a*): posteriorly this leads into a double chamber, the compartments being separated from each other by a slight longitudinal partition; transversely there exists a series of chitinous *trabeculae* (*tr*). A closing apparatus is present but this is best described in the abdominal spiracles. There is no spiracle present on the meso- or metathorax, neither is there any indication of this structure in the last three abdominal segments. Each of the other abdominal segments bears laterally and somewhat anteriorly a pair of spiracles of normal structure (*i.e.* not biforous). Each (Fig. 9 *b*) consists of a spiracular opening (*o*) which leads into the *atrium* (*a*) and this extends posteriorly into an oval chamber across which are arranged 6 or 7 transverse *trabeculae* (*tr*). At the inner end of the atrium is the closing apparatus consisting of a chitinous bow (*ch*) the base of which unites to form a chitinous band around the trachea. The longer chitinous

arm extends posteriorly, while the short one lies in an antero-lateral position. Occlusor muscles are attached to these rods and function in opening and closing the spiracular opening.

6. The Pupa (Fig. 10).

The pupa is soft, of creamy white colour, and is capable of active movement when touched or exposed to changes in temperature. It varies in length from 2·0–2·5 mm., and usually lies on its side within the pod. The head is bent ventrally and the elongate rostrum extends to the abdomen. As in the adult the size of the rostrum indicates the sex of the pupae. A few scattered bristles are visible in the anterior region, but the arrangement of these does not appear to be characteristic. The pupal integument is densely covered with minute papillae. The antennae (*a*) extend from the base of the rostrum in a latero-anterior direction. The legs are folded ventrally, the prothoracic (1) and mesothoracic (2) legs in a more or less anterior position, while the tarsi of the metathoracic (3) legs extend posteriorly to the 7th abdominal segment. The tips of the elytra extend to the 6th abdominal segment, the hind wings being completely concealed by the elytra. The abdomen has 10 distinct segments, the 10th segment being extremely rudimentary, appearing as a mere tubercle. The 9th abdominal segment terminates in two prominent caudal spines.

7. Life-History.

Hibernation.

Apion ulicis hibernates as the adult and in this stage has been beaten from gorse bushes through the winter. It does not hibernate normally within the pod as stated by Bargagli(2). Examination of debris and soil beneath the bushes for hibernating weevils yielded negative results but close observation of the branches revealed adult specimens—their greyish colour resembling small buds—at the points where buds and spines leave the branches. During a spell of sunshine these adults become more active and are easily observed. Dissection of the reproductive organs of about 500 females periodically during the winter months showed the absence of sperms in the spermatheca and also revealed the immature condition of the ovaries, thus confirming field observations that mating had not taken place. Further, it was not until the end of February when the weevils became more active and were observed nibbling the branches and young shoots, that any appreciable quantity of food was observed in the alimentary canal.

Mating. On March 2nd sperms were first found in the spermatheca of a single female but, despite daily observation, mating was not observed in the field until March 26th. Later, in April and May it was commonly observed. Pairing was not witnessed after the end of May. Prior to mating, the male with rostrum held in a ventro-posterior position follows the female, eventually seizing it by placing the claw of one of its anterior legs on the anterior ridge of the prothorax. Continuing this action for some time the male eventually mounts and copulation takes place. Within a glass tube or cage the male and female pair at intervals, but under natural conditions, from the comparative ease with which the female can remove the male by pushing under the spines of the gorse plant, it would appear that a single pairing normally occurs. A virgin female, after a single copulation had taken place, was found to have its spermatheca filled with sperms. In many cases it was observed that the male, during copulation, scraped the scales from off the back of the female with its tarsal claws, thus resulting in a black fertile female. This accounted for the quantity of black females found in the field and all such females were found to be fertilised. It was, however, later noticed that the removal of scales did not occur during every copulation. The period from the date of mating to the time of oviposition varied from 30–42 days.

Oviposition. The gorse did not come into flower in Harpenden until mid-April, and pods of any appreciable size (the anthers and calyx of flower being still retained) were not observed until mid-May. Daily observations of the gorse for oviposition were continued throughout May and the first instance was observed on May 11th. According to Goureau (8) oviposition took place in February and March in S. France. As illustrated in Fig. 11 the female first bores a hole in the pod with its rostrum. A large series of counts taken indicates that no particular portion of the pod is chosen for oviposition while frequently the weevil bores through where the calyx still surrounds the pod. It was clearly observed, however, that the weevil prefers a young pod and oviposition ceases on bushes where the pods have become hard and black. The time taken for boring the hole varied between 1 and 5 hours, feeding naturally took place during this operation, for on removal of the rostrum the mandibles were observed still at work. After the withdrawal of the rostrum, the female turns around and orientates itself by means of its anal end until the ovipositor is placed within the hole (Fig. 12). Occasionally the ovipositor is placed within a hole recently made by another female, while on the other hand, several attempts at orientation were observed to be

entire failures. One particular instance of what might be termed "love's labour lost" was witnessed in which the female, after spending from 2–7 p.m. boring the hole, attempted without success, for half an hour to place its ovipositor within the hole in the pod and it finally walked off. Instances of this kind usually result in the eggs being deposited outside the pod but as will be seen later, eggs thus laid do not develop. There is no attempt whatever to close up the hole in the pod which can clearly be seen under the binocular microscope. As the pod develops these holes become closed and it is very difficult to find a trace of a hole in a mature green pod, while in a mature dark pod detection is impossible. The eggs are laid in batches within the pod (Fig. 13), the normal number per batch being 6–8 eggs. More than one batch of eggs frequently occurs within a single pod; these are probably instances of two females ovipositing in the same pod and even in the same hole. The number of eggs per pod obtained from counts taken from a large number of pods varied from 1–23. Oviposition continued at Harpenden during May until early August. Experiments arranged to ascertain the number of eggs laid by a single female were rendered void owing to the fact that all the eggs were not placed within the pods.

Incubation.

The incubation period was 26 days (± 4) during which the egg changes from an elongate yellow form and assumes a spherical pearly white appearance. The form of the embryo within the egg can be seen through the delicate chorion about the 20th day. The embryo is curled back upon itself, the head and anal region practically touching each other. On hatching the chorion splits in the mid-dorsal region of the embryo, the latter pushing the chorion over both head and anal end as it emerges, and eventually tugging itself away from the remaining delicate chorion.

Larval Period.

The young larva, after emergence, wriggles its way to the base of the seed, where the soft funicle of the seed affords its first food. The larva shows definite negative phototropism and, because of this fact and also that frequent disturbance is detrimental to the development of the larva, investigations with a view to ascertaining the number and nature of the instars gave unsatisfactory results. Further, after the larvae had pierced a hole in the seed coat, in several instances it entered the seed and was thus lost to observation. It is, however, certain that the first moult takes place on the 9th or 10th day after emergence and the final

moult occurs just prior to pupation. At the last moult the comparatively large head capsule is discarded and remains close to the pupa. The entire larval period is 45 days (± 5).

Cocoon formation.

Despite the fact that the larvae is enclosed within the gorse pod and sometimes within the seed coat, during its last instar it proceeds to make a cocoon. The cocoon consists of a brown glutinous material forming a distinct chamber closely surrounding the pupa. Mature larvae were observed making cocoons and it was seen that a light brown material exuded anally. Dissection proved that the material practically filled the alimentary canal and offered a marked contrast to the green contents of the alimentary canal of younger larvae. The substances exuded spasmodically indicating definite expulsion by the larva which removed the material from the anus by means of its mandibles. In the region of the mouth-parts the excreted mass evidently received a salivary secretion, for the entire surface of the mouth-parts was bathed in a colourless fluid which welled up at intervals and was mixed with this anal secretion. The mixture was then arranged into a cellular chamber with distinct walls and a roof which eventually encloses the larvae. The necessity for this cocoon appears to be obscure unless it assists in hindering the passage of parasitic Hymenopterous larvae which have been observed isolated from enclosed pupae. It may also be necessary to maintain a constant humidity.

Pupal Stage.

Continuous examination of pods from the Harpenden common throughout the summer, yielded the first pupa on July 8th. Pupae predominated in the pods during the latter part of July and in August, a few were found as late as October 9th. Under laboratory conditions the pupal stage was 10 days (± 2). The pupae remain white until the last few days of the pupal period. Pigment first appears in the eyes and rostrum, later it develops in the thorax, the coxae, apices of the femora, in the tibia and the tarsi. On opening pods weevils with their elytra and abdomen still white have walked out.

Adults.

The number of adults per pod varies considerably and the results of numerous counts became so interesting that it was decided to examine in detail 500 infected pods taken at random on the Harpenden Common. The normal number of individuals per pod was 4·6, the number varying

from 1–16. The normal arrangement of the adult weevils within the pod prior to emergence is seen in Fig. 14. The weevils are usually laterally placed, and when numerous the method of packing is extraordinarily efficient. The partitions of the cocoons can also be seen in the photograph. The adult weevils emerge from the pod when the pod dehisces on fine sunny days. The crackling of gorse pods in the sunshine is a familar sound in all gorse areas. Normally the seeds are hurled into the air when the pods burst, so that in the case of infected pods, the weevils are similarly thrown out and immediately become active. Despite the possession of strong biting mandibles the adults are incapable of emerging from the pod by their own efforts. This fact was strongly suggested by the discovery in April and May of quantities of unopened pods containing dead adult weevils. The fact has also been fully proved by retaining quantities of unopened infected pods in the laboratory, and on examination after several months later no adults had emerged, but when the pod was opened mechanically the weevils immediately became active. Further observations in the field show that a certain number of pods do not open naturally for some reason or other, they are retained on the plant or fall to the ground. Such pods have been collected during the Winter and Spring, and some have yielded unattacked seeds; some, dead or moribund weevils and others, weevils which became very active as soon as the pod was opened. A sample of old unopened pods was examined in May 1927 and these yielded live adults obviously from the 1926 generation. The female reproductive organs of these weevils when examined were found to be quite immature, while normally the females were ovipositing in the field. In view of the fact that the gorse pod depends on bright sunny weather in order to dehisce, it is probable that as the result of a wet Summer and Autumn, large numbers of weevils will suffer the fate of being thus imprisoned. Weevils emerging normally in late Summer and Autumn can be found quite active on the gorse plant on sunny days, but at the first indication of frost they become sluggish and are difficult to see on the plant. On very warm days in Spring and Summer they will readily take to the wing. It may be of interest to note that they strongly exhibit positive phototropism.

8. Damage.

The actual damage the adult weevils do appears to be negligible, it is a mere browsing and puncturing of the spines and softer portions of the plant.

From the 500 collected pods previously mentioned the damage caused by the larvae to infected pods was ascertained. It was found that 69·4 per cent. of these pods had their entire contents devoured by the larva of *Apion ulicis*, while 18·6 per cent. had a single whole seed remaining, 10 per cent. had two entire seeds and 2 per cent. had three seeds still intact. It is especially interesting to note the economy of food by the larvae which occurs under certain conditions. The number of seeds in a normal pod varies from 4–7. From the data collected it was seen that 3 larvae could devour the entire contents of a normal pod, while in several instances as many as 16 larvae had developed and produced apparently quite normal adults. A few of these were slightly smaller in size but there was no marked difference.

It is obvious that to ascertain a normal percentage of pod infestation counts would have to be taken after oviposition had ceased. This was done on three occasions at Harpenden when 200 pods were collected at random and on examination it was found that 88, 77 and 82 per cent. of the pods were infected respectively.

9. Survey of the Damage in Great Britain.

It is clear that to secure absolute figures for the percentage pod infestation of *Apion ulicis*, for any given area or for Great Britain as a whole, would involve far more work than a single person could undertake.

It was, however, felt desirable to ascertain the percentage pod infection *possible* under conditions in Great Britain, and further to secure some indication of regional distribution.

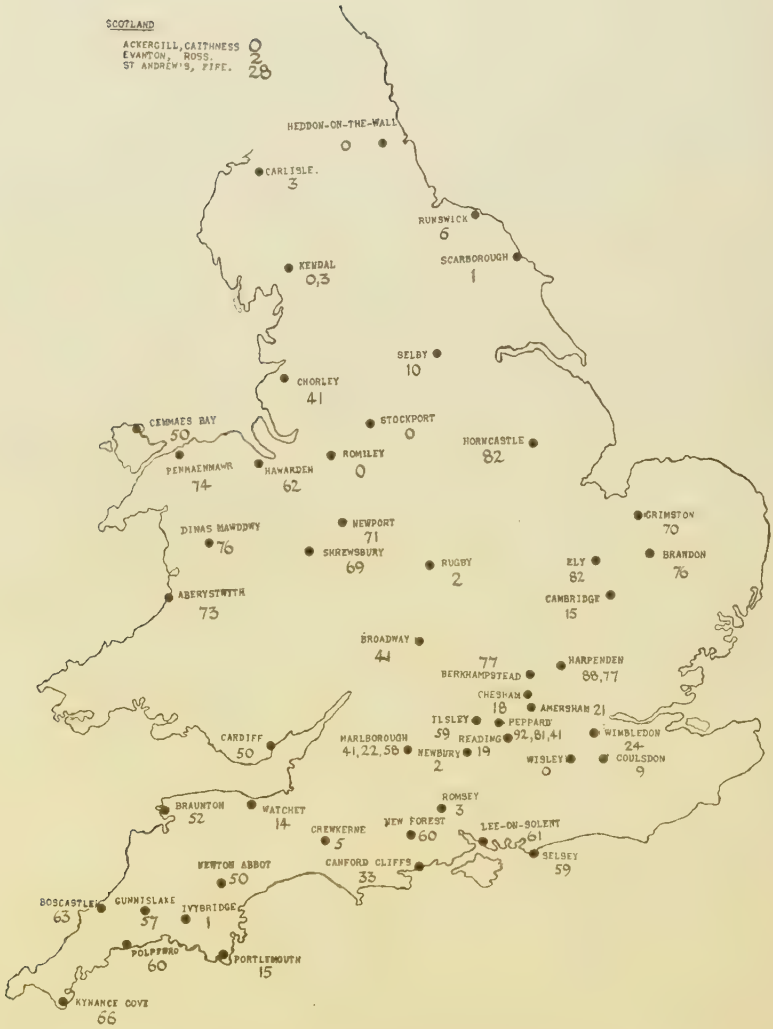
Survey of infestation of Apion ulicis *in Great Britain.*

No.	County	District	% pod infection	Remarks
1	Caithness:	nr Wick, Ackergill	0	Extreme N. Scotland. 30 % L
2	Ross:	Evanton	2	30 % L
3	Fife:	St Andrews...	28	—
4	Northumberland:	Heddon-on-the-Wall	0	32 % L
5	Cumberland:	Carlisle	3	—
6	Westmoreland:	Scout Scar, Kendal ...	3	6 % L
7	„	Paddy Lane, Kendal ...	0	—
8	Yorkshire:	Runswick nr Whitby ...	6	21 % L near seashore
9	„	Scarborough	1	7 % L
10	„	nr Selby, Riscal Common ...	10	—
11	Lancashire:	Chorley	41	32 % L
12	N.W. Derby:	Stockport	0	Alt. 800 ft. 16 % L
13	Anglesey:	Cemmaes Bay	50	—
14	Carnarvon:	Penmaenmawr	74	Alt. 700 ft.
15	Flintshire:	Hawarden	62	—
16	Cheshire:	Romiley	0	Alt. 450. 1 % L pods black with soot

No.	County District	% pod infection	Remarks
17	Lincoln: Horncastle	82	2 % L
18	Merioneth: Dinas Mawddwy	76	—
19	Salop: Newport	71	3 % L
20	„ S.E. Shrewsbury, Fetch Hill ...	69	25 % L
21	Warwick: Rugby	2	15 % L
22	Norfolk: Grimston	70	—
23	Suffolk: Brandon Common	76	—
24	Cambridge: 1½ miles S. Ely	82	3 % L
25	„ Cambridge University Farm	15	Taken from gorse hedge, no other gorse for several miles
26	Cardigan: Aberystwyth	73	—
27	Worcester: Bayliss Hill, Broadway ...	41	5 % L
28	Hertford: Harpenden	88	—
29	„ „	77	—
30	„ „	82	—
31	„ Berkhampstead	77	—
32	Buckingham: Chesham	21	48 % L
33	„ Amersham	18	—
34	Berks: Isley	59	10 % L
35	Essex: Epping	85	6 % L
36	Bucks: Ibstone	18	6 % L
37	Oxford: Peppard Common	81	—
38	„ „ „	92	Taken from old bushes, not known to have been burnt. 2 % L
39	„ „ „	41	Adjacent to the foregoing, bushes periodically burnt
40	Berks: Reading	19	—
41	„ Padworth...	64	—
42	„ Newbury	2	12 % L
43	Wilts: N.W. Marlborough	58	12 % L
44	„ S. Marlborough	42	—
45	„ S.W. Marlborough	22	13 % L
46	Surrey: Wimbledon Common	24	21 % L
47	„ Wisley	0	55 % L
48	„ Esher Common, nr Coulsdon ...	9	68 % L. Some *Apion* half eaten
49	Somerset: Doniford, nr Watchet ...	14	11 % L
50	„ Crewkerne	5	30 %
51	Hampshire: Romsey	3	56 % L
52	„ New Forest	60	29 % L
53	„ Lee-on-Solent	61	—
54	Dorset: Canford Cliffs	33	21 % L. Some *Apion* half eaten
55	Devon: Braunton	52	19 % L
56	„ Newton Abbot	50	—
57	„ Ivybridge	1	36 % L
58	„ Portlemouth	15	27 % L
59	Cornwall: Gunislake	57	—
60	„ Boscastle	63	22 % L
61	„ Polperro	60	—
62	„ Kynance Cove	66	—

L = pods also attacked by lepidopterous larvae.

An organised survey has been made possible through the kind assistance of a number of persons to whom the writer is greatly indebted. Samples of 100 pods each gathered at random over gorse areas from



Map. Distribution of *Apion ulicis* in Great Britain.
(Figures indicate percentage pod infection.)

54 selected districts through Great Britain, have been secured. From this number of pods there will be a probable error of ± 10 per cent. infestation. These pods have been examined for *Apion ulicis* by the writer and the results are indicated in the table and on the map. It is then seen that as high as 92 per cent. pod infestation has occurred, but it should be noted that this particular infestation in Oxfordshire was on old bushes which as far as could be ascertained were not known to have been burnt, at least for very many years. Adjacent to these bushes was another area of gorse which had periodically been burnt and here the percentage was reduced to 41. The habit of burning gorse in Great Britain, then, undoubtedly decreases the efficiency of *Apion ulicis* in destroying gorse seeds, and no doubt accounts for many of the low percentages recorded. Further, the presence of Lepidopterous larvae reduced the percentage of attack by *Apion ulicis*, for in many cases the caterpillars had devoured the entire contents of the pod, larvae or pupae of the weevil included. Several cases of half eaten pupae were noticed. It is interesting to note that from the samples received no really high infestation of *Apion ulicis* was recorded from the North of England and Scotland. This result needs confirmation.

The infestation on Harpenden Common has been under observation by Dr Imms for some years, and it is stated that in certain years it has been practically impossible to secure a sample of sound seeds. A few counts were made in July 1926 by H. T. Pagden, and though the numbers counted were small in comparison with the present year, the pod infestations taken from 7 different counts averaged 77 per cent.

10. Tests on Economic Plants.

Before an insect can be introduced into a new country it is, of course, essential that the particular insect should undergo most critical tests on all the plants of economic importance that there is the slightest possibility of it attacking. This aspect of the work has therefore received primary attention. The method adopted for all work of this kind is to subject the insect concerned to a "starvation test" when death of the insect on the particular economic plant concerned is the only criterion that will justify further consideration of that species. It is felt that the selective faculty of the insect cannot be relied upon in this matter, for one cannot assume that if an insect is specific in its host plant in the field, it will remain so under all conditions. Not only are "starvation tests" carried out in this country, but all insects successfully standing

these tests in this country will be submitted to similar tests in their new environment abroad.

The technique used to test *Apion ulicis* can be grouped into three sections.

I. *To test if eggs of* Apion ulicis *laid outside the pods could develop.*

As previously mentioned it was found that under unfavourable conditions *Apion ulicis* laid its eggs outside the pod, on the branches and elsewhere. It was very important to ascertain if these could develop. Eggs thus laid were collected and arranged on the outside of the pods in the field, the eggs being protected with muslin bags. Three series of 50 eggs each were then tested in turn. On each occasion in two or three days the eggs had shrivelled to almost unrecognisable masses. A similar series of experiments was carried out with eggs normally laid within the pod and carefully removed and placed on the outside of the pod in the field. These all suffered a similar fate to the preceding. It was next desirable to test if newly hatched larvae could penetrate the pods from the outside. All newly hatched larvae placed on the outside of pods perished within a day or so.

From these results it was obvious that the gorse pod played an essential part in the life-history of the weevil, for without it development was impossible. Thus it followed that the only economic plants to be considered in the tests were pod-forming species of the Leguminosae.

II. *To test whether* Apion ulicis *would oviposit in pods of other leguminous plants.*

The pod-forming plants which have been considered are: Broom (*Cytisus* (*Sarothamnus*) *scoparius* Link.), Lupin (*Lupinus*), Broad Bean (*Vicia faba*), Kidney Bean (*Phaseolus vulgaris*), Garden Pea (*Pisum sativum* L.), Lucerne (*Medicago sativa*) and Wild White Clover (*Trifolium repens* L.). It was found that the factor of captivity could be ignored in the case of *Apion ulicis*, for females readily oviposited in a gorse pod within a test tube. It was noted, however, that under similar conditions *Apion ulicis* would not oviposit in pods of other plants. Three cages of growing plants of each of the above species were arranged and 30 ovipositing females together with 10 males were put in each cage. A cage of *Ulex* was kept as control. Examination of pods after 2 months interval resulted in practically every gorse pod being infected whereas no sign of oviposition in the pods of the other plants was witnessed. At this

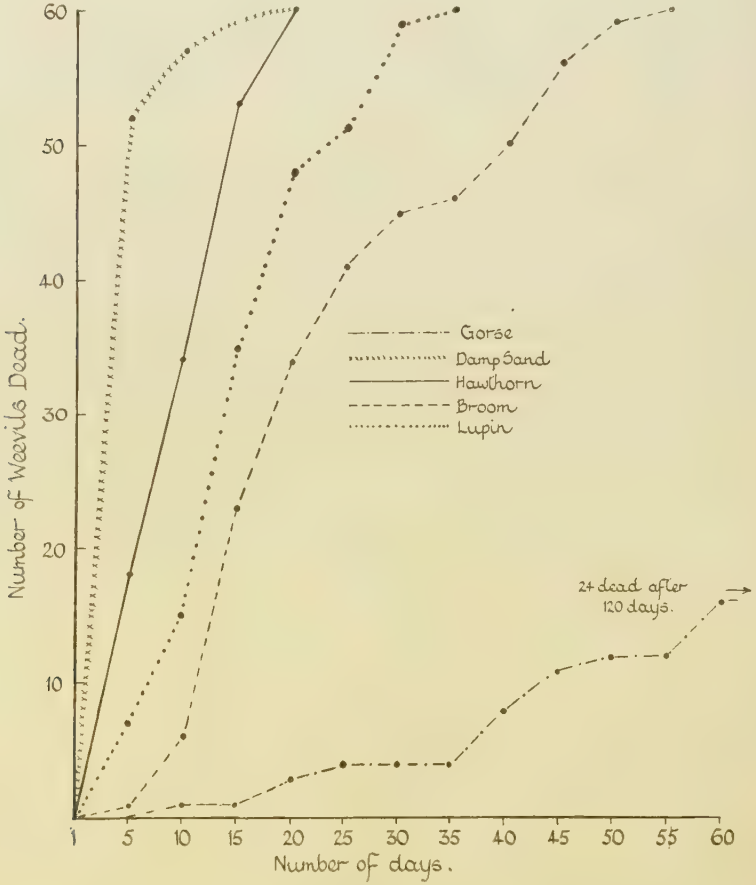
date no live adults could be found on the "tested" plants, while 28 females and 5 males were still alive on the gorse. This test was further elaborated in that both eggs and larvae of different ages were placed within the pods of tested plants. Pods of Broom, Lupin, Broad Beans, and Peas only were found practicable for the tests. A series varying from 10–20 pods of each plant was experimented with: eggs, and larvae of varying sizes, were put in each pod. Examination of pods later showed that while in some instances slight nibbling of the seeds of the tested plant had taken place, not a single larvae developed to the pupation stage.

III. *To test if adult weevils can survive on other leguminous plants.*

To secure comparable results the method shown in the photograph (Fig. 15) was adopted[1]. Sprigs of the plants concerned were cut and enclosed within a lamp-glass, the top of which was covered with a muslin cap; the stalks projected into a test-tube of water. Each plant was tested in triplicate, 20 weevils being placed in each. Counts were taken every 5 days, and the results have been plotted in Graphs I and II. In Graph I the weevils used were those of the 1926 generation and thus the tendency of the weevils to die off on the gorse is observed. There is, however, a striking difference between the death rate on gorse and that on other host plants. The host plants tested were confined to lupin and broom, because at the time when this experiment was commenced there was not a sufficient supply of the other plants available. Graph II gives the results of a larger series of experiments, where the weevils used were those secured from the pods of gorse before they had fed on their natural host plant. It was felt that these tests would give more reliable results. It is regretted that after the 45th day an accident to the tray of experiments rendered further procedure impossible. It is fortunate, however, that the experiment was sufficiently far advanced to give significant results and the final termination of the curves can be approximately assumed. Repetition was impossible owing to the fact that frost soon occurred and the weevils commenced hibernation. Actual nibbling of the plants of lupin and broom was observed, but it is evident from the curves that this was not beneficial to the weevils. In fact from the position of the "damp sand" and "hawthorn" curve there is a strong indication that some of these host plants may actually be detrimental to the welfare of the weevil. It is quite obvious that under the conditions

[1] The technique is that used by C. T. Gimingham in research on insecticides and a detailed description of the same will be shortly published.

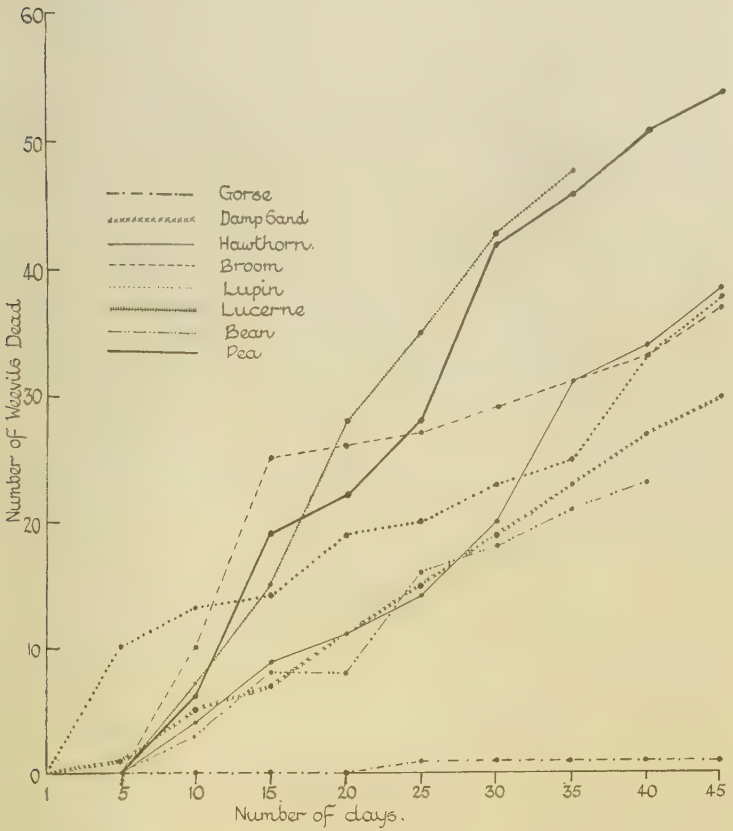
of these tests these plants cannot serve as food for *Apion ulicis*. It is interesting to note that *Apion ulicis* has been recorded from *Ulex nanus*, and Bargagli(2) states that it has been observed on *Genista tinctoria*.



Graph I. Death-rate of *Apion ulicis* (old specimens) on economic plants.

Regarding the occurrence of *Apion ulicis* on broom (*Cytisus scoparius*) it should be stated that on Harpenden Common there is a small area of broom among the gorse. Periodical examination and beating of these bushes with a view to ascertaining the presence or otherwise of the

weevil, have yielded entirely negative results; despite the fact that *Apion ulicis* is in abundance on the adjacent gorse bushes.



Graph II. Death-rate of *Apion ulicis* (newly emerged) on economic plants.

11. Parasites.

One of the most important points in biological control is the separation of a beneficial insect from its parasite, or hyper-parasite as the case may be. Thus the greatest possible care has to be taken lest the parasite be introduced into the new environment along with its host. Such a step might result in entire failure of the attempt. It is, of course,

also essential that parasites of the insect about to be introduced should not already exist in the new environment.

Observations have been made regarding parasites of *Apion ulicis* and a few have occurred in practically every locality. An estimation of the percentage infestation has been made at Harpenden; the 500 pods previously referred to afforded the following data: 9 per cent. of the infected pods were infected with parasites, the number of parasites per pod varying from 1–8. The actual percentage of *Apion ulicis* parasitised was only 4. The degree of parasitism at Harpenden was quite the normal of other districts investigated.

The parasites proved to be all of one species[1]. This species was originally described by Goureau(8) as *Semiotus apionis*; the genus *Semiotus* Wlk., it may be added, has now become a synonym of *Semiotellus* Wstw. Dr Waterston, who kindly identified these parasites, however, states that while the parasites obtained from *Apion ulicis* agree perfectly with Goureau's description of *Semiotus apionis*, they do not belong to the genus *Semiotellus*, so that the generic position of this species will need to be ascertained.

There are three other records of parasites of *Apion ulicis*: *Pteromalus pirus* Wlk. and *Eulophus ulicis* Perr. both recorded by De Gaulle(3), and *Semiotus brevipennis* Walk. bred by Goureau (Dours. Cat. 102). All these records are from France.

12. Summary.

1. The present study of *Apion ulicis* Först. is in reference to the use of this weevil in the control of *Ulex europaeus* in New Zealand: its synonymy and geographical distribution are dealt with.

2. The external morphology of the egg, larva, pupa and imago of *Apion ulicis* have been studied, special attention being devoted to the mouth parts of both adult and larva. The male and female reproductive organs are also described and figured.

3. The details of its life-history and feeding habits are given and an account of the damage caused by both adult and larva is included.

4. A survey of 62 districts in Great Britain has been organised and as high as 92 per cent. pod infection has been observed.

5. Primary attention has been given to the possibility of *Apion ulicis* attacking economic plants. It was found that only leguminous plants need be considered, and of these oviposition only occurred in

[1] Dr Waterston has since identified the species as *Splintherus leguminum*, Ratz.

pods of *Ulex europaeus*. Tests to ascertain the ability of *Apion ulicis* to thrive on other leguminous plants gave entirely negative results.

6. A parasite identified as *Splintherus leguminum*, Ratz. has been bred, a 4 per cent. parasitism was estimated. Three other records of parasites of *Apion ulicis* are quoted.

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14. EXPLANATION OF PLATES XV—XVII

PLATE XV.

Fig. 1. *Apion ulicis* Först. (female). ×20.

Fig. 2 *a*. *Apion ulicis*: mouth parts of adult (dorsal view). ×300. Lettering as Fig. 3.

Fig. 2 *b*. *Apion ulicis*: mandible of adult. ×300. *ad m*, adductor muscle; *ab m*, abductor muscle; *c*, condyle, *gm*, ginglymus; *ph b*, pharyngeal bracon.

Fig. 3. *Apion ulicis*: ventral aspect of mouth parts of adult. ×300. *sm*, submentum; *mt*, mentum; *mx p*, maxillary palp; *l p*, labial palp; *lg*, ligula; *lc*, lacinia; *p*, palpifer; *cd*, cardo; *sg*, subgalea; *st*, stipes; *mx*, maxilla; *mn*, mandibles.

Fig. 4. *Apion ulicis*: male reproductive organs (dorsal view). ×36. *a*, aedeagus; *a g*, accessory gland; *e d*, ejaculatory duct; *t*, testes; *tr*, transfer apparatus; *r*, chitinous rod; *v d*, vasa deferentia; *m*, longitudinal muscles.

Fig. 5. *Apion ulicis*: female reproductive organs (dorsal view). ×36. *a g*, accessory gland; *b c*, bursa copulatrix; *m*, vaginal muscles; *o*, ovariole; *od*, oviduct; *sp*, spermatheca; *sp d*, spermatic duct; *vit*, vitellarium; *vg*, vagina; *t*, terminal filament; *u*, uterus; *r*, chitinous rod.

PLATE XVI.

Fig. 6. *Apion ulicis*: mature larva (lateral view). ×36. *I*, prothoracic segt.; *II*, mesothoracic segt.; *III*, metathoracic segt.; *h*, head capsule; *p sc*, prescutal lobe; *sc*, scutal lobe; *scl*, scutellar lobe; *abd I*, 1st abdominal segt.; *abd X*, 10th abdominal segt.; *sp*, spiracle; *pl g*, pleural groove; *hlp*, hypopleural fold; *ep*, epipleural lobe; *st*, sternellar fold; *pl*, pleurites.

Fig. 7. *Apion ulicis*: mouth parts of larva. ×135. *a*, antenna; *cl*, clypeus; *e*, labrum; *ep*, epistoma; *ep l*, epicranical plate; *e s*, epicranial suture; *fr*, frons; *mn*, mandible.

Fig. 8. *Apion ulicis*: mouth parts of larva (ventral view). ×135. *A*, maxilla; *cd*, cardo; *st*, stipes; *sgl*, subgalea; *gl*, galea; *lc*, lacinia; *f*, palpifer; *mx p*, maxillary palpus. *B*, *mt*, mentum; *l p*, labial palp; *pm*, prementum; *sm*, submentum.

Fig. 9. *Apion ulicis*: spiracles of larva. ×325. *A*, Biforous spiracle of pro- and metathorax; *B*, Abdominal spiracle; *a*, atrium; *ch*, chitinous bow; *o*, spiracular opening; *pr*, peritreme; *t*, tracheae; *tr*, trabeculae.

Fig. 10. *Apion ulicis*: pupa (female) ventral view. ×36. *a*, antenna; 1, prothoracic legs; 2, mesothoracic legs; 3, metathoracic legs; *abd IX*, 9th abdominal segt.; *c s*, caudal spine; *el*, elytra.

PLATE XVII.

Fig. 11. *Apion ulicis* (female) boring hole in gorse pod prior to oviposition.

Fig. 12. *Apion ulicis*: female with ovipositor in gorse pod.

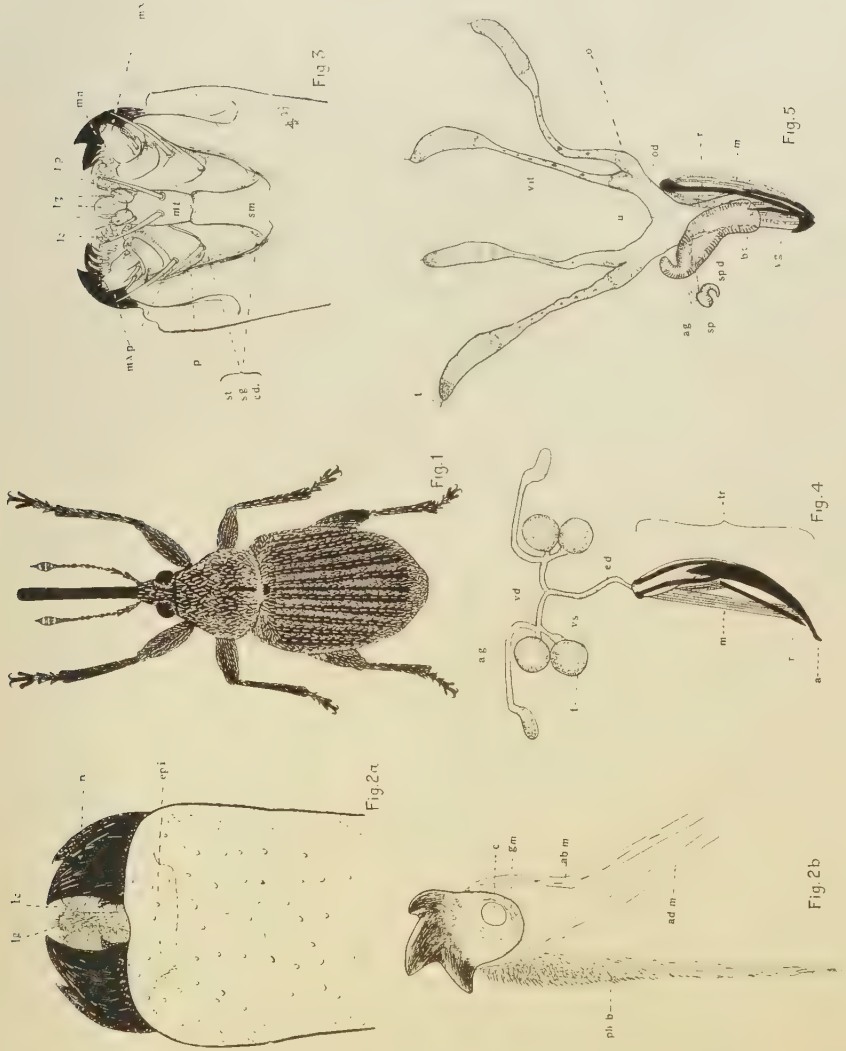
Fig. 13. *Apion ulicis*: batches of eggs *in situ* within gorse pod.

Fig. 14. *Apion ulicis*: adults within gorse pods just prior to emergence; remains of cocoons visible.

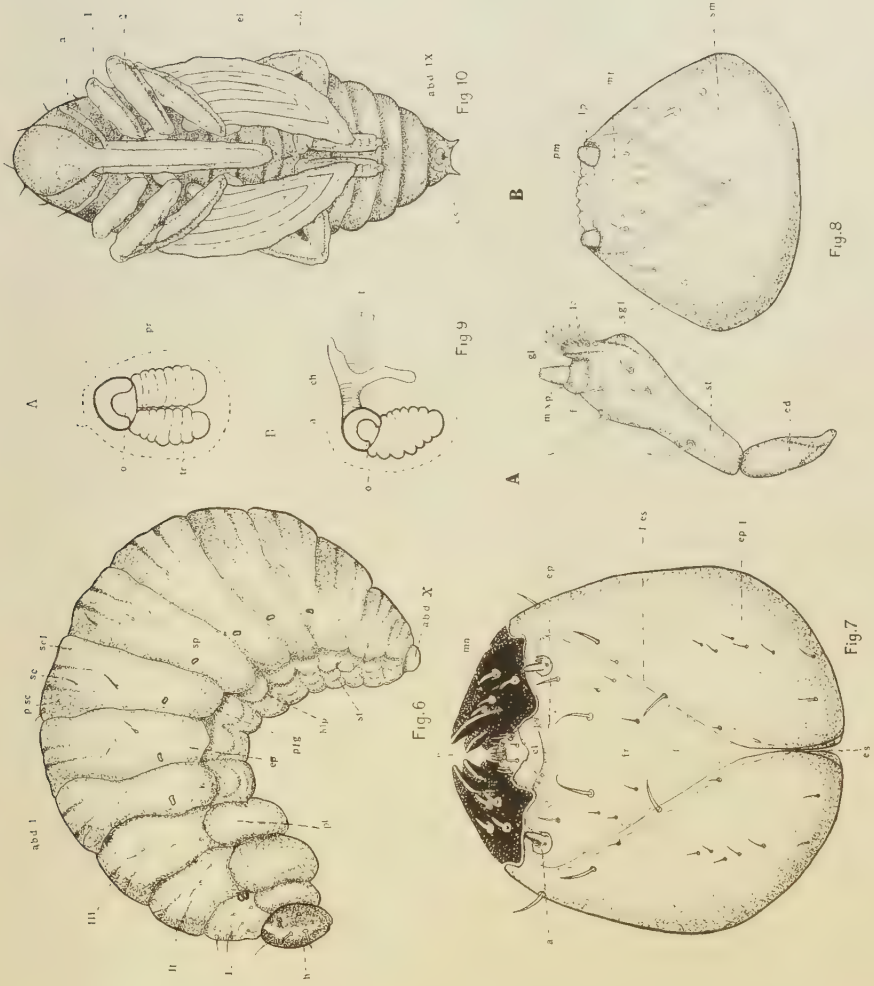
Fig. 15. Portion of insectary with tray of "Starvation test" experiments *in situ*.

Figs. 11 to 15 are from photographs taken by V. Stansfield.

(*Received December 8th*, 1927.)



MALDWYN DAVIES.—The Bionomics of *Apion ulicis* (pp. 263–286).



MALDWYN DAVIES.—The Bionomics of *Apion ulicis* (pp. 263–286).

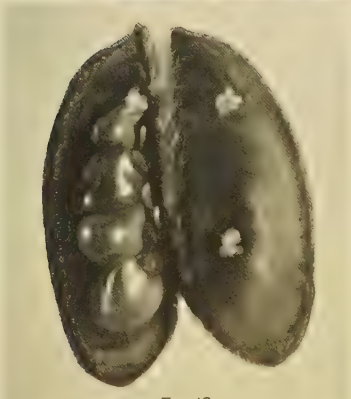


Fig.13


Fig.14

MALDWYN DAVIES.—The Bionomics of *Arion ulicis* (pp. 263–286).

ON THE LIFE-HISTORIES AND ECONOMIC STATUS OF CERTAIN CYNIPID PARASITES OF DIPTEROUS LARVAE, WITH DESCRIPTIONS OF SOME NEW LARVAL FORMS

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(*From the Zoological Laboratory, Cambridge University.*)

(With 12 Text-figures.)

CONTENTS.

Introduction.

Despite several papers of recent years treating of the biology and bionomics of individual Cynipids our knowledge of parasitic Cynipids is still extremely scanty.

In view of the economic importance of some of them and the variety of interesting and significant larval forms exhibited during their life cycles much more attention will assuredly be paid to this group. In this paper an endeavour has been made to study some of the commoner species of Cynipids parasitising dipterous larvae. Probably the best known Cynipid of this kind is *Cothonaspis rapae* (Westd.) belonging to the great sub-family *Eucoilinae*. It is a most effective parasite of that devastating garden and field pest, the cabbage root maggot (*Hylemyia brassicae* Bouché).

The commonest forms found parasitising carrion-feeding dipterous larvae belong to the genus *Figites* of the sub-family Figitinae (Dalla

Torre) and to the genus *Kleidotoma* of the sub-family Eucoilinae (Dalla Torre).

Although Graham Smith(6) records Cynipids of the genus *Diranchis* (Först.) bred from the puparia of saprophagous maggots the writer obtained none belonging to this genus during one season's work. The weather was very inclement, however, during the whole of the time occupied by the work. I wish to thank Prof. J. Stanley Gardiner, F.R.S., for according me facilities in his laboratories to carry out this investigation and for help in other ways. The writer is indebted to Dr Hugh Scott for placing his wide systematic knowledge of insects at my disposal, thus greatly facilitating my work.

Acknowledgments are also due to the following gentlemen for assistance rendered at various times; Dr D. Keilin, Mr R. C. L. Perkins, F.R.S., Dr G. S. Graham-Smith, Mr F. R. Petherbridge, Dr K. M. Smith and Mr A. T. Paskett.

Working Methods.

Cynipid parasites ovipositing in saprophagous dipterous larvae were obtained by exposing meat such as liver or "lights" in shallow circular metal trays about 2 ft. in diameter, and allowing it to putrefy. Cynipids were attracted during the early putrefactive stages and could be found crawling in the cracks and crevices of the meat. Owing to their limited powers of flight they were easily caught. After the parasites had been identified they were transferred to glass breeding tubes about 1 in. in diameter and from 6 to 9 in. long, which were stoppered by close fitting corks. The corks were bored and pieces of fine wire gauze inserted into slits made in the corks so that the gauze fitted across the holes, and while admitting air denied egress to the parasites. The Kleidotomids especially were extremely difficult to keep confined in the breeding tubes not only on account of their very small size but also because of their aptitude to squeeze through crevices in the corks smaller even than themselves. It was found necessary to seal the cork down with sealing wax as this type of parasite was found capable of boring through soft material such as putty.

Into the tubes containing the parasites were slipped small quantities of decaying meat resting on small pieces of paper. The latter facilitated the subsequent manipulation and removal of the meat when it became semi-liquid owing to the action of the host larvae. On to each piece of meat in the breeding tubes was placed a small number of eggs of some host Dipteron. The species of host most commonly used were: *Calliphora*

erythrocephala Meig., *Lucilia sericata* Linn., *Lucilia caesar* Linn., *Musca domestica* Linn., *Sarcophaga carnaria* Linn., *Hydrotaea dentipes* Fab.

The host eggs soon hatched and the parasites quickly began ovipositing in the young larvae.

The parasites appeared quite indifferent to the species of dipterous maggot presented to them provided its natural medium was putrefying meat. With a laboratory temperature of about 60° F. 2 or 3 hours were sufficient to ensure oviposition.

The time and date of oviposition having been recorded on a label gummed to the base of the breeding tube the parasites were transferred to fresh material in another tube. Never more than two female parasites were used for oviposition in a breeding tube. The parasitised maggots would develop quite satisfactorily provided the tube was washed out occasionally and a new supply of suitable food placed therein. The maggots could thus be taken out and dissected at whatever stage they were required. Near the time of pupation the parasitised maggots were supplied with a layer of fine damp sand in which they could pupate. Petri dishes were found useful for this purpose. In this way many parasites were successfully reared. In the case of *Cothonaspis rapae* (Westd.) the Cynipid parasite of the cabbage root maggots (*Hylemyia brassicae* Bouché) young cabbage plants were grown in large size plant-pots and allowed to become infested with the young larvae of *H. brassicae* by enclosing the adult flies above the plants in muslin-topped glass cylinders. Adult *Cothonaspis* parasites bred out from puparia of *H. brassicae* collected in the field were then introduced into the breeding cylinders. Oviposition usually took place. The parasites could also be induced to oviposit when they were confined in glass breeding tubes which contained pieces of cabbage root containing maggot embedded loosely in soil.

All the figures of larval forms included in this paper were made from living specimens using a camera lucida. A considerable amount of time and patience were required to make good stained and permanent mounts of the early stage larvae owing to their fragility and only a few were made. Borax carmine proved the best stain employed for this purpose.

The Life-History of *Cothonaspis rapae* (Westd.).

Cothonaspis rapae has long been known as a parasite of *Hylemyia brassicae* the cabbage root maggot and is one of the best known and most common Cynipids.

The following is a description of *C. rapae* (Westd.) given by Kieffer and Dalla Torre (13).

"Schwarz, glänzend. Flagellum der Antenne pechbraunrot, beim ♀ $\frac{3}{4}$ so lang wie der Körper, kräftig, 3. Glied kaum um die Hälfte länger als das 4., das 5. ein wenig länger als das 4., die letzten 8 eine deutlich abgesetzte Keule bildend. 6. Glied länger als das 7., und meist dünner als dieses. Antenne beim ♂ länger als der Körper, 3. Glied ein wenig länger und nicht viel dünner als das 4. Scutellum hinten und seitlich dicht runzlig, Napf meist eirund, Gruben gross, breiter als lang. Flügel glashell, gelblich rauchgrau angehaucht; Adern scherbengelb oder braun; 1. Abschnitt der Radialis mehr als halb so lang wie der 2., der 3. fast so lang wie der 1. und 2. zusammen, gebogen; Cubitalis vollständig. Coxae, Trochanteren und mehr oder weniger die Femora proximal, Tibien und Tarsen pechbraunrot. Abdomen linsenförmig, etwas länger als Kopf und Thorax zusammen; Haarbinde breit, schmutziggrau. L. 2·75–4 mm."

As will be seen from the foregoing description the sexes are easily distinguished by the lengths of the antennae. Pairing was not observed although both sexes were kept together for several days in breeding tubes and on suitably enclosed cabbage plants. Unfertilised females appeared to be capable of laying eggs which developed normally but the sex of the resulting progeny was not investigated. Possibly the refusal to mate may be a reaction of the parasite to captivity. When kept in confinement on a suitable member of the Brassica family both sexes of the parasite appeared to be capable of living for a considerable time. One female survived a month but 14 to 18 days was the normal period of life when supplied with plenty of material for oviposition.

The power of flight of *C. rapae* is much better developed than the cynipid parasites of the carrion feeding Diptera to be described later. When about to oviposit the female Cynipid crawls down the stalk and oviposits in the larvae as they lie in or on the roots. The soil around the stem of a cabbage badly attacked by *H. brassicae* is seldom pressed closely around it and the movements of the ovipositing female are thus facilitated.

The latter are only capable of ovipositing in very small larvae either of the first or second instar. Tests with large larvae, eggs or puparia always gave negative results. Thus the efficiency of the parasite, great though it already is, would be more than doubled if the whole of the larval stage was susceptible to parasitisation by the Cynipid. Only a relatively short period of the host's life cycle is vulnerable to attack. Darkness appears to be an essential pre-requisite for oviposition. *C. rapae*

could never be induced to oviposit in a breeding tube unless the piece of root containing the maggots was loosely embedded in soil.

This appears to indicate that maggots in the stems and leaves of the host plants, a not exceptionable nidus in certain plants, would be immune from the attacks of this Cynipid even if their position rendered oviposition physically possible.

In view of this negative phototropism of the female parasite during oviposition it was never possible to witness the latter. One egg was usually left in the haemocoel of the maggot. Two have occasionally been found by the writer but never more. In the latter case one of the parasites soon dies after hatching and they have been now and again found in various stages of degeneration. Occasionally the deposition of two eggs in a host was fatal to both host and parasites but these may have been cases of superparasitism. The minute yolkless egg is of the pedunculate type common to Cynipids and resembles that of *F. anthomyiarum* (Fig. 5) except that there is never a constriction in the body of the egg. *C. rapae* has a very great egg laying capacity and will continue ovipositing for about 10 days after a 2-days period of maturation immediately following emergence. The ovaries are large and resemble those of the Figitid in Fig. 4. This type of ovary is also found among the Kleidotoma. The period of incubation of the egg in *Cothonaspis* is about 6 days, although a low temperature will lengthen this period considerably.

The primary larva. The writer was fortunate to witness the eclosion of the primary larva and Fig. 1 represents this.

The chorion (*CH.*) is ruptured anteriorly by the larval head and posteriorly by its relatively long cauda which is curled towards the head prior to hatching.

Hypermetamorphism occurs during the development of *C. rapae* as is the case for all Cynipids whose life cycles are known. The primary larva is a eucoiliform. This is a type of larva first described by Keilin and Pluvinel(12) in a study of *Eucoila keilini* (Kieff.), an endoparasite of *Pegomyia winthemi* Mg. and its chief distinguishing features are the presence of three pairs of long thoracic processes and a long cauda. The larva on hatching makes considerable use of these thoracic processes to escape from the egg membrane. The living larva was kept under close observation in distilled water for 5 minutes. Although the thoracic processes were waved vigorously to and fro little or no motion of the larva as a whole was observable. Although the conditions of haemocoelic life were by no means reproduced it suggests that these processes have no utility for locomotory purposes. When free from the egg membrane

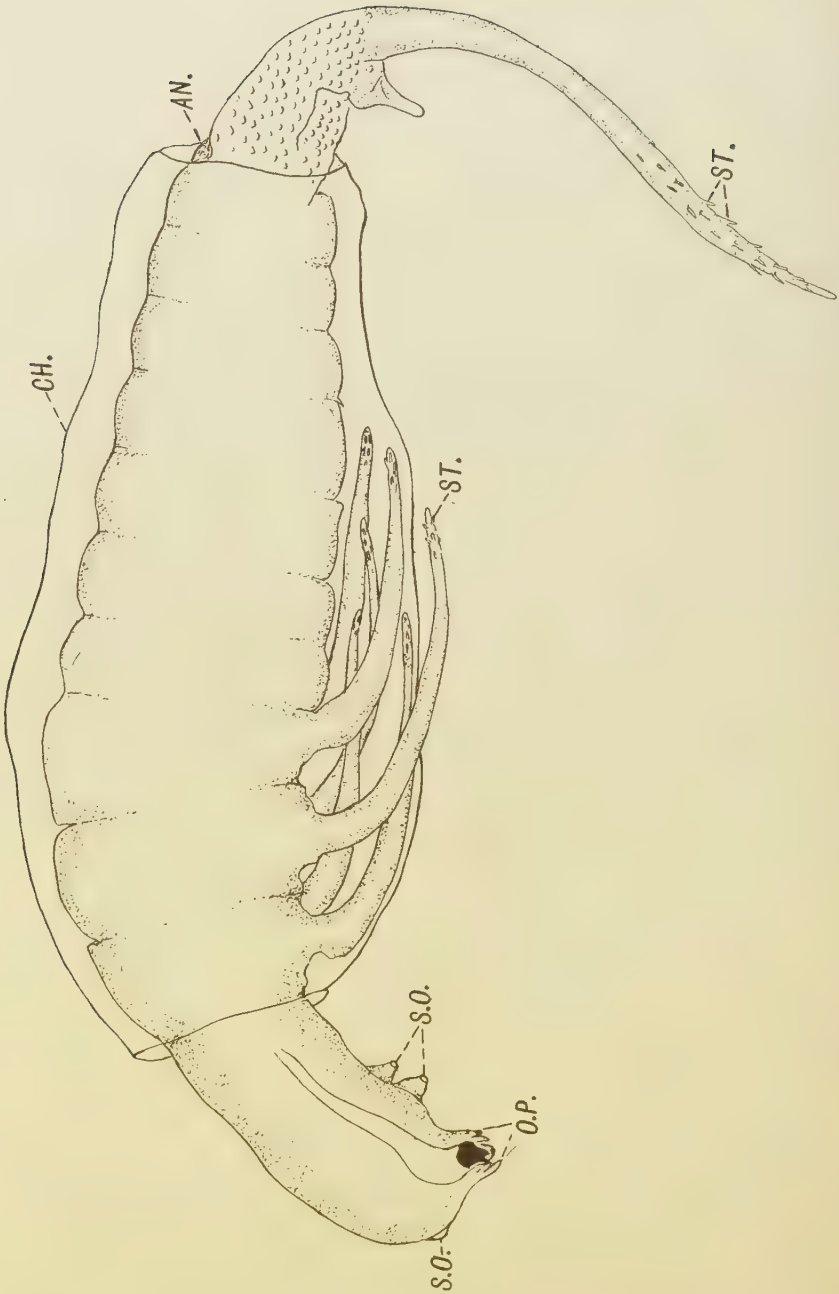

Fig. 1. The eclosion of the larva of *Cothonaspis rapae* (Westd.) from the egg (protopod stage). ×254 diams. Drawn *in vitro* with camera lucida. *AN.*, anus; *CH.*, chorion; *O.P.*, oral papillae; *S.O.*, sensory organs; *ST.*, setae.

the primary larva measures about 0·7 mm. long. The head is somewhat elongate and bears anteriorly a sensory process. On the ventral surface of the head there are two prominent projections with transparent extremities which also appear to subserve a sensory function (Fig. 1, *S.O.*). Antero-ventrally is a large rounded projection on whose surface lies the oral opening. The latter is surrounded by several papillae (Fig. 1, *O.P.*). Inside the mouth a small sclerite is distinguishable but there are no indications of mandibles. The structure of the mouth indicates beyond all doubt that the method of feeding is entirely haemophagous.

The condition of the gut affords evidence that feeding takes place during this instar. The mouth opens into a somewhat wide pharynx which soon becomes constricted into the aesophagus. The head is clearly demarcated from the body region and exhibits no indications of segmentation. The segmentation of the body region is fairly distinct. First there are three clearly marked thoracic segments each with a pair of long processes furnished distally with minute setae (Fig. 1, *ST.*). Seven segments are distinguishable in the abdominal region but at least two other segments go to form the rest of the abdomen and the cauda. The anus opens on the dorsal surface at the posterior margin of the 7th segment (Fig. 1, *AN.*). Its size and position suggests a resemblance to the first stage larva of *Charips* (*Allotria*), a Cynipid hyperparasite through a Braconid, of aphides (Haviland (10)).

Between the posterior margin of the 7th segment and the base of the cauda the chitinous integument appears to be covered with a scale-like ornamentation. Ventrally in this region there is a prominent projection which is a fairly constant feature of eucoiliform larvae. The long cauda is armed distally with small setae which are specially long and numerous near the tip. As already mentioned the cauda is of considerable use in assisting the larva to escape from the egg. In carefully stained preparations of the larva the gut can be faintly seen but nothing of the nervous system could be defined with certainty. There is a complete absence of a tracheal respiratory system, and how these creatures respire provides an interesting problem in insect metabolism. It has been suggested in the case of other endoparasitic larvae that gaseous interchange takes place cutaneously but nothing is really understood about the process.

The primary larval stadium only lasts about 2 or 3 days and then an ecdysis occurs which reveals a larva not essentially different from that of the first instar. There is, however, a slight increase of size and the abdominal segmentation is more distinct. Although it was not

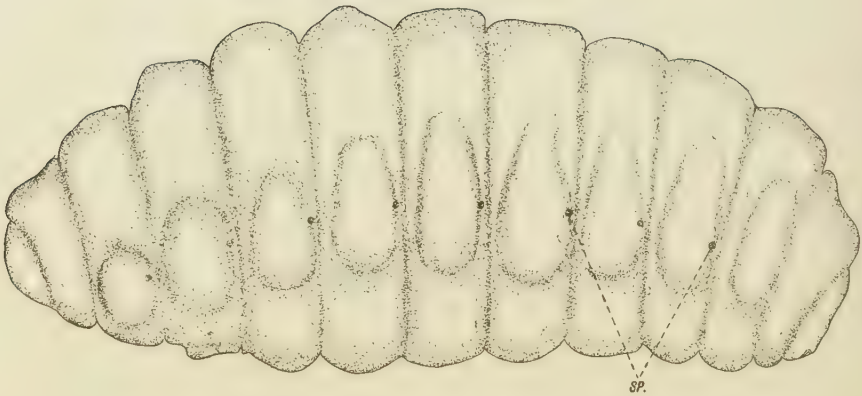

Fig. 2. The full grown larva of *Cothonaspis rapae* (Westd.) in side view. × 40 diams. *SP.*, spiracles.

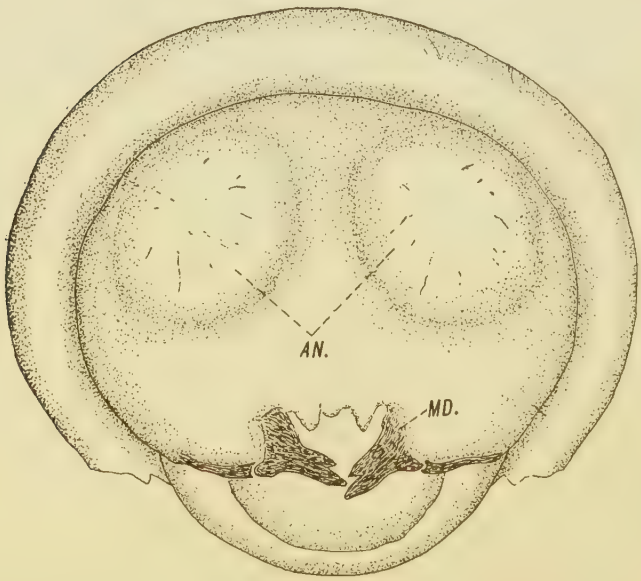

Fig. 3. The anterior view of the cephalic segment of the full grown larva of *Cothonaspis rapae* (Westd.). × 160 diams. *AN.*, voluminous antennal papillae; *MD.*, mandibles.

actually observed there is little doubt that the next stage in the life cycle of *Cothonaspis rapae* was represented by a polypodeiform type of larva most probably similar to that figured for *K. marshalli* (Fig. 11).

In later instars a tracheal respiratory system gradually develops and the cauda is gradually lost.

The full grown larva of this parasite consists of a somewhat chubby legless hymenopterous grub of the usual type measuring about 3 mm. in length (Fig. 2). It possesses a cephalic and 13 body segments. Each of the latter from the 3rd to the 10th inclusive possess a pair of spiracles situated laterally near the anterior margin of each segment. Each body segment from 2 to 10 inclusive is provided laterally with a pair of voluminous papillae immediately behind the spiracles when the latter are present. The integument is smooth and devoid of hairs. The cephalic segment is provided with a pair of narrow bi-dentate mandibles (Fig. 3, *MD.*). There are two rounded swellings on the dorsal anterior aspect of the cephalic segment similar to but not so large as those described in *Eucoila keilini* (12). Keilin and Pluvinel regard them as homologous with antennae.

Duration of life cycle. Two main periods of emergence of *Cothonaspis rapae* were noticed during the season. First those individuals which had overwintered in the puparia of *Hylemyia*[1] *brassicae* Bouché emerged in May and produced a second generation which emerged in late August and in September.

The total length of the life cycle of *Cothonaspis rapae* varied in 53 cases from a minimum of 70 days to a maximum of 111 days with an average of 92 days. Seven individuals gave an average length of larval life of 55 days. This latter figure is based on cases where dissections disclosed a transforming larva or one on the point of so doing. Hibernation takes place in the larval stage. The pupal stage is thus seen to occupy a very large part of the total life cycle.

Parasitised larvae of *H. brassicae* developed much slower than healthy ones and in their later stages showed a tendency to premature pupation if removed from their pabulum.

[1] The writer has followed Smith (15) in referring to the cabbage root fly under the generic name of *Hylemyia* and not under the older and less accurate one of *Chortophila*.

The Control Value of *C. rapae* for the Cabbage Root Maggot *H. brassicae*.

The incidence of parasitisation of the Cynipid *Cothonaspis rapae* (Westd.) on *H. brassicae* is about 25 per cent. for the Cambridge district, including the big cabbage growing area around Gamlingay. This figure is based on an examination of 3800 maggots and puparia of the host. Smith(15) gives 30 per cent. as the percentage of parasitisation of *H. brassicae* by *C. rapae* in Lancashire and Cheshire.

The factor which considerably restricts the value of *C. rapae* as an effective control of *H. brassicae* is the very short period of the life cycle of the host which is open to attack. As already mentioned, only the first two larval instars of the host provide suitable material for oviposition.

The high natural rate of parasitisation, however, leads one to believe that the encouragement of this parasite might be productive of good results in still further reducing the damage caused by *H. brassicae*. It is also worthy of mention that in the material of *H. brassicae* examined by the writer the rate of parasitism recorded for the Staphylinid beetle *Alechara bilineata* (Gyll) was almost as high as that recorded for the Cynipid.

The Life-History of *Figites anthomyiarum* Bouché.

Systematic. Various species of the sub-family Figitinae have been recorded as being bred from dipterous larvae, but hitherto little or no attention has been paid to their life-histories and economic value as parasites. The species dealt with in this paper is *Figites anthomyiarum* Bouché, of which Kieffer and Dalla Torre(13) give the following description.

"Schwarz. Gleicht dem *F. scutellaris* (S. 88). Antenne beim ♂ lebhaft rotgelb, 1. Glied schwarz; Glieder des Flagellum walzenförmig, Endglied braun. Antenne beim ♀ pechbraun, kürzer, Glieder des Flagellum kugelig. Prothorax und Mesopleure gestrichelt. Mesonotum gerandet. Scutellum runzlig. Mediansegment uneben, jederseits mit erhöhtem Stigma. Flügel glashell, Adern gelb. Beine lebhaft rotgelb; Tibia des Hinterbeines am Proximalende und Krallenglieder braun. Abdomen eiförmig, zusammengedrücht, glänzend, glatt; 1. Segment gürtelförmig und gefurcht; 2. Tergit bei ♂ und ♀ vorn gestrichelt. L. 2·7 mm."

Seasonal prevalence. The species of the genus *Figites* now under consideration was first observed in the meat trays in June but possibly they often appear earlier.

Graham Smith (6) has a record of an undetermined Figitid which was bred out in May in a case where the possibility of spring infection had been eliminated.

F. anthomyiarum was present in greater or less numbers on the meat trays until about September 10th when the extremely wet weather which prevailed for the rest of the month stopped their activities for the remainder of the season.

Proportion of the sexes. The males which are usually somewhat smaller than the females were never caught by the writer on the meat trays or in the vicinity of the latter. Although the males bred out from parasitised material were fairly numerous, there seemed to be such a predominance of females as would suggest that a certain amount of parthenogenesis occurs. The act of mating was not observed.

Length of adult life. When confined in breeding tubes of a type described in an earlier section of this paper and given plenty of material for oviposition the maximum life of a female was about 8 or 9 days. They appear to derive all necessary nourishment from the juices produced by the activity of the maggots in the meat. Females which were kept under similar conditions but refused maggots for oviposition will live up to 15 days provided they have access to a little decaying meat which has been infested with maggots. Males in captivity lived only about 5 days.

Flight. Both sexes fly little and the female soon becomes incapable of flight after spending several hours crawling in the cracks and crevices of the meat. Neither sex is quick to take to the wing and the females are easily caught in their natural habitat.

Oviposition. Those female parasites which were bred in the laboratory and afterwards used for breeding purposes required about 2 days for maturation before they would commence ovipositing. The parasite only oviposited in young larvae of the first or second instar and seemed to prefer them immediately after they were hatched. No other stage in the life cycle was attacked. They were never seen to attack a larva openly. They prefer to direct their long and curved ovipositors into larvae which were almost or completely enclosed in their pabulum. At the same time the parasite herself would be almost hidden from view in some fissure of the meat.

The compressed abdomen of the Figitid rendered it admirably adapted for getting into very small crevices and the act of oviposition very frequently takes place while the insect is lying on its side. Immediately after the ovipositor has been inserted in the host the latter

becomes quiescent for a period varying from 1 to 2 minutes owing probably to the injection into the larva of a potent secretion. The latter in that case would undoubtedly be the product of the two glands shown in Fig. 4, *AC.* with their reservoir *RES.* The length of time during which the ovipositor remained in the host never exceeded a minute. *F. antho-*

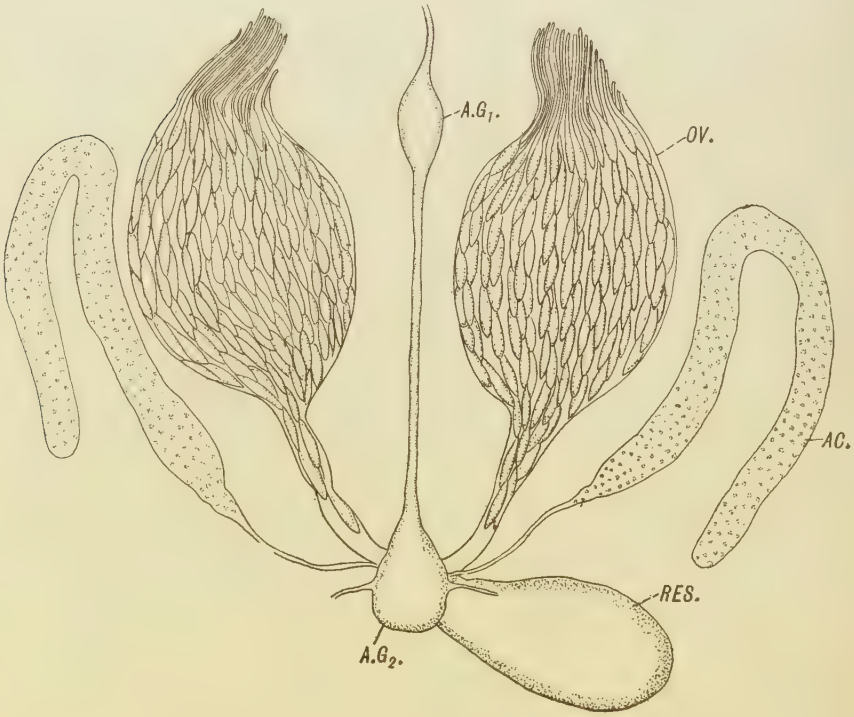

Fig. 4. The reproductive system of *Figites anthomyiarum* Bouché. ×95 diams. Female. *AC.*, acid gland; $A.G_1$., $A.G_2$., first and second abdominal ganglia; *RES.*, acid gland reservoir; *OV.*, ovary.

myiarum possesses two large ovaries containing a large number of minute eggs (Fig. 4, *OV.*).

Never more than two eggs were laid in a maggot at a single oviposition, and though both parasites would hatch, one invariably died and occasionally both. One egg was found far more frequently than two. The stimulus to oviposit is undoubtedly the chemotropic reaction caused by the odour of decaying meat, as will be shown in a later experiment.

The parasite does not appear to aim at inserting the ovipositor into any pre-arranged spot, so long apparently as the egg is placed in the haemocoelic fluid of the host the object is accomplished.

Fig. 5.

Fig. 6.

Fig. 5. The egg of *F. anthomyiarum* Bouché. ×500 diams. Drawn *in vitro* with camera lucida.

Fig. 6. The primary larva of *F. anthomyiarum* shown *in situ* in the egg about 6 hours before eclosion. ×275 diams. Drawn *in vitro* with camera lucida. *AN.*, anus; *CH.*, chorion.

The egg. The egg is of a typical Cynipid type with elongate body and a pedicel as long as the body (Fig. 5).

Its average measurement is about 0·20 × 0·02 mm. The chorion is externally smooth. A curious feature of the egg is the constriction of

the body about its middle into a definite waist which is not however quite an invariable feature.

Period of incubation. The period of incubation is about 2 or 3 days. The pedicel soon begins to degenerate after development begins. Fig. 6 shows the primary larva of *Figites anthomyiarum in situ* in the egg about 6 hours before hatching takes place.

The chorion has been partially removed so that a better view of the larva could be obtained.

The primary larva. The newly hatched larva of *F. anthomyiarum* measures about 0·45 mm. long and 0·13 mm. at its broadest point (Fig. 7). It may be considered as a modified eucoiliform type with reduced thoracic processes and cauda. Its affinities to the eucoiliform type of larva are further shown by the unpaired process on the ventral surface of the last segment near the base of the cauda and by the sensory process on the ventral surface of the head (Fig. 7, *S.O.*) which are usually found on eucoiliform larvae.

The head is distinct from the body, somewhat elongate, and bears a resemblance to that of the primary larva of *Cothonaspis rapae* (Westd.).

The mouth is situated antero-ventrally on a circular prominence which is surrounded by a number of papillae. Internally, in the oral region some kind of minute sclerite can be discerned, but there can be little doubt from its structure that the mouth is capable only of a sucking function and that during this stadium at least the sole food of the larva consists of the haemocoelic fluid of the host. Coming to the body region, the segmentation is more clearly defined than that of the primary larvae of *C. rapae* and *E. keilini.*

Thirteen segments can be counted including the last segment which gives attachment to the cauda. The three thoracic segments are rather larger than the succeeding abdominal ones and each bears a pair of appendages.

The pair of appendages on the prothoracic segment are extremely short. The pair on the mesothoracic segment possess a short branch near the tip and are the longest of the three pairs. Behind the thoracic segment there follows 10 clearly defined abdominal segments. The larval integument, particularly on the dorsal surface, is covered by chitinous spines and setae. The anus is not of the enlarged type found in certain eucoiliform larvae and also described by Haviland(10) for the primary larva of *Charips* (*Allotria*).

When newly dissected out of the host the primary larva is of a translucent white colour and beyond the faint outline of the gut

Fig. 7. The primary larva of *F. anthomyiarum* Bouché ×366 diams. Drawn *in vitro* with camera lucida. *A.N.*, anus; *S.O.*, sensory organ.

nothing can be seen of the internal structure. After staining, preferably with borax carmine, other internal structures become apparent. The ventral nerve cord consists of a thick band extending almost the entire length of the larval body. There is a supra- and a sub-oesophageal ganglion mass and constrictions appear along the cord at segmental distances, dividing the latter into ganglia, of which 10 could be distinguished. The dilator muscles of the pharynx are also visible in well stained specimens. There are no indications of a tracheal system. The primary larval stage lasts about 5 or 6 days (when the temperature is above 60° F.) and then an ecdysis occurs and the eucoiliform larva gives place to one of an entirely different type.

The second stage or Polypodeiform Larva. The second stage larva is elongated in form and measures usually about 1 mm. long (Fig. 8). The first point to which attention should be drawn is that compared with the primary larva, it appears to have undergone a reduction in the number of segments. In the latter there are 13 apparent segments in addition to the head whereas the former possesses only 11 body segments. These latter probably have a true metameric value since the same number of body segments are found in the full grown larva. The second feature, one of considerable embryological interest, is that the first 10 body segments each possess a pair of processes which occupy the position of, and in every way appear to be, the rudiments of true appendages (Fig. 8, *R.A.*).

These pairs of processes diminish gradually in size from before backwards. Reference will be made later in this paper to the ontogenetical significance of the appearance of this entirely new type of larva among the endoparasitic Hymenoptera.

The head segment possesses a rather conspicuous looking chitinous endoskeleton (Fig. 8, *C.E.*) which shows up prominently through the integument. The mouth is similar to that of the primary larva in having no mandibles and food in this stage is still ingested in the fluid form. Anteriorly about the mouth the larva is armed with a pair of long papillae (Fig. 8, *O.P.*) about whose precise nature and function the writer is uncertain but they may be both tactile and gustatory.

On the ventral surface of the head there is a conspicuous sensory organ consisting of a chitinous projection surmounted with a transparent tip (*S.O.*). The general surface of the integument is smooth and devoid of hairs or setae.

The last segment bears ventrally a stout cauda whose characteristic position is almost at right angles to the long axis of the larval body.

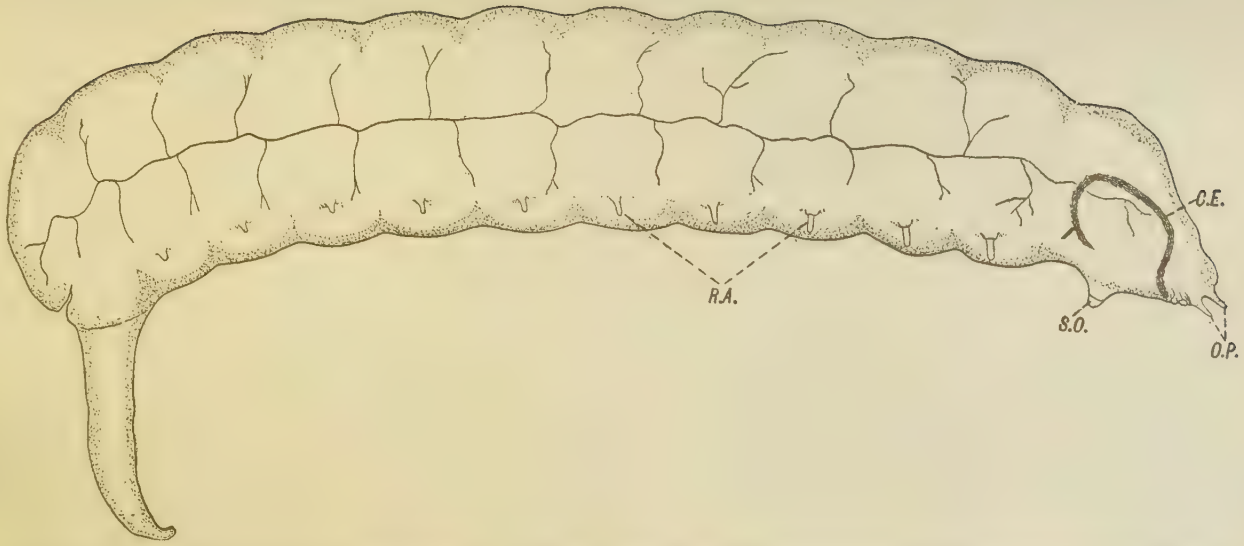

Fig. 8. The polypodeiform larva of *F. anthomyiarum*. ×175 diams. Drawn *in vitro* with camera lucida. *C.E.*, chitinous endoskeleton of the head; *O.P.*, oral papillae; *R.A.*, rudimentary appendages; *S.O.*, sense organ.

Internally an apneustic tracheal system has developed which extends almost the entire length of the larva. It consists of two lateral trunks each of which gives off dorsal and ventral branches usually at segmental distances. No trace of spiracles could be found.

The alimentary canal shows up clearly and globules of food material can be seen in it. The separation between the mesenteron and the proctodaeum was also plainly evident. The nerve cord was essentially similar to that seen in the primary larva. The second larval instar lasts about 10 days and is succeeded by stages in which the pairs of processes characteristic of this stage have disappeared.

There is a gradual shortening of the cauda at each successive moult and a peripneustic tracheal system develops.

A description of the full grown larva of *F. anthomyiarum* has already been given by Bouché(4). The latter worker obtained his material from the puparia of *Anthomyia dentipes* and *A. floralis*. It possesses 12 segments, and pairs of spiracles are present on segments 2 to 10 inclusive. There are no appendages and the mouth is armed with a pair of bidentate mandibles.

The pupa is of the usual exarate hymenopterous type which gradually darkens as the pupal period advances and this stage does not call for any special comment. The pupal stage lasts about 20 days and the adult Figitid emerges through an irregular hole usually near one end of the host puparium.

Hibernation takes place in the larval stage in the host puparium.

Duration of Life Cycle. The average length of the life cycle for individuals of the summer generation is 60 days, but is much longer for members of the overwintering generation.

There are two summer broods and possibly a third in favourable seasons. The duration of the pupal stage in the overwintering generation was not observed.

The Life-History of *Kleidotoma marshalli* (Marshall) and an Undetermined Species of the Genus *Kleidotoma*.

Very little is known of the biology of the minute members of this difficult group beyond the fact that some species are known to be endoparasitic in dipterous larvae. The species treated in this paper are *K. marshalli* and an undetermined Kleidotomid. Kieffer and Dalla Torre(13) give the following description of *K. marshalli*.

"Schwarz. Antenne beim ♀ so lang wie Kopf und Thorax zusammen, beim ♂ um die Hälfte länger; 2. Glied etwas rundlich, dick, 3½ mal so

lang wie das 4., die übrigen dicker als lang; Keule abgesetzt; 1. Keulenglied fast so lang wie die 3 vorigen Glieder zusammen, ein wenig kürzer als das 2., 3. Keulenglied fast so lang wie die 2 vorhergehenden zusammen, distal etwas kegelförmig; Keule so lang wie der Rest des Flagellum; 3. Glied beim ♂ gebogen, nicht viel länger als das 4. Glied. Scutellum dicht längsgestreift; Napf schmal, vorn scharf zugespitzt.

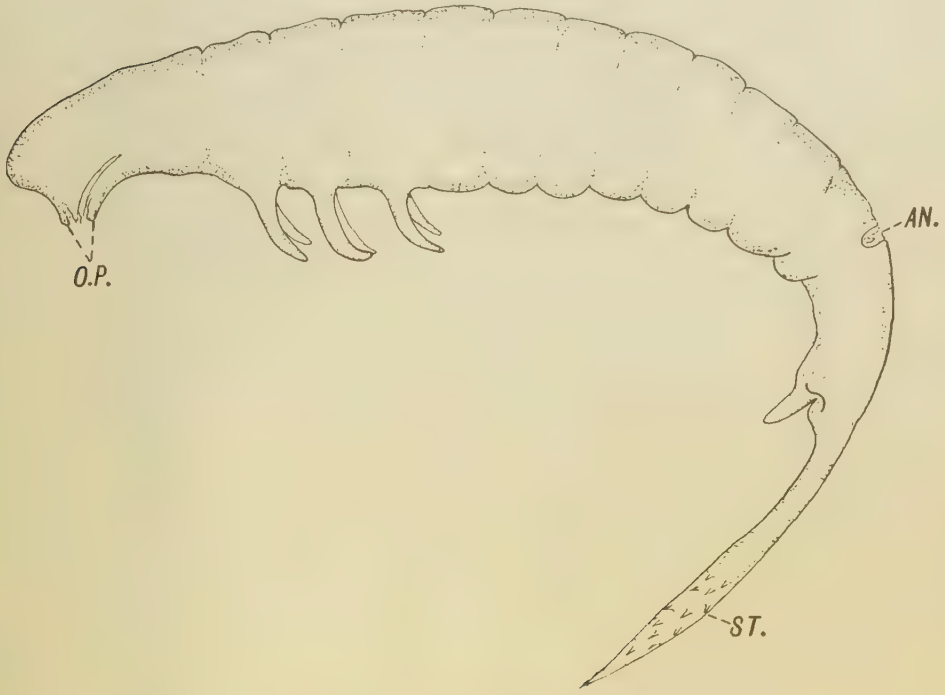

Fig. 9. The primary larva of *K. marshalli* (Marshall). ×327 diams. Protopod stage. Drawn *in vitro* with camera lucida. *AN.*, anus; *O.P.*, oral papillae; *ST.*, caudal setae.

Flügel glashell, an der Spitze wenig tief, aber deutlich ausgerandet; Haarsaum lang; Adern scherbengelb, 2. Abschnitt der Radialis um $\frac{1}{4}$ kürzer als der 3.; Radialzelle proximal und distal geschlossen, verlängert, schmal, mehr als 2 mal länger als breit. Beine scherbengelb, Coxae und Proximalende der Femora schwarz gestreift. Abdomen länger als der Thorax; Haarbinde oben unterbrochen reinweiss. L. ♀ 2, ♂ 1·5 mm."

Kleidotomid species first appeared in the meat trays in June and

could be found among the carrion until the wet cold weather in the middle of September suspended their activities as it did in the case of

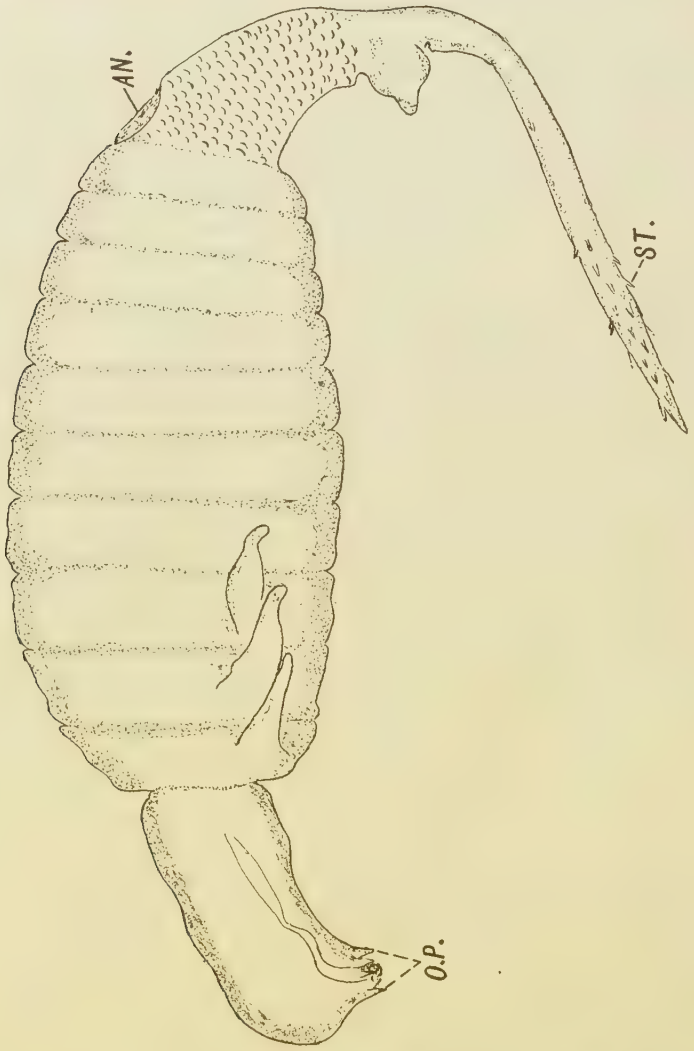

Fig. 10. The primary larva of an undetermined Kleidotomid. ×587 diams. (Protopod stage.) Drawn *in vitro* with camera lucida. *AN.*, anus; *O.P.*, oral papillae; *ST.*, caudal setae.

F. anthomyiarum. Kleidotomids are much smaller than Figitids but are quite as numerous. Their powers of flight appear to be even more limited than that of the latter genus. No male Kleidotomids were

observed on the meat trays. The length of life of the female adult in the laboratory was shorter than that of *F. anthomyiarum*, for when supplied with plenty of material for oviposition none lived longer than 6 days. The act of oviposition was performed in a manner essentially similar to that of the Figitine species described above. Only very small larvae were selected for parasitisation. The egg is of the same type as that of *F. anthomyiarum* but is smaller and does not possess the somatic constriction so very frequently found in the latter. The ovaries, although smaller, do not differ essentially from those of *F. anthomyiarum* or *C. rapae* and are well stocked with minute ova. The period of incubation lasts about 2 days. The primary larvae of both *K. marshalli* and the unidentified species were of the eucoiliform type. They differ from the primary larva of *C. rapae* in that the thoracic processes are much shorter and are devoid of setae.

The position of the mouth in both cases is more ventral than that of *C. rapae* and there are also no sensory structures on the ventral surface of the head segment.

The primary larva of *K. marshalli* (Fig. 9) is very elongated, measuring 0·40 mm. long, and appears to possess one more segment than the primary larva of the undetermined Kleidotomid.

The latter is shorter and plumper and possesses a longer head (Fig. 10). It measures only 0·25 mm. in length. The mouth in both species is of the same shape as that of *C. rapae* and also resembles it in being devoid of mandibles and surrounded by papillae. It is obviously suctorial in function. The cauda is long and prominent in both species and covered with setae at the distal end. The anus is of the enlarged type not uncommon in early stage cynipid larvae.

The first instar larva in both species of Kleidotomids lasts about 10 days, after which an ecdysis occurs and the primary larvae in both cases change into the polypodeiform larva shown in Fig. 11.

The polypodeiform larva of a Kleidotomid measures about 1·0 mm. long and possesses a large cephalic segment followed by 11 very clearly defined segments, to the last of which is attached a long cauda. Here again, as in the case of *F. anthomyiarum*, we get that apparent reduction in the number of segments in passing from the protopod to the polypod larval phase. The head segment contains a conspicuous endoskeleton (Fig. 11, *C.E.*). The head bears anteriorly a suctorial mouth guarded by two processes which may be homologous with antennae (*O.P.*). As in the preceding stage mandibles were absent. There is a prominent sensory projection on the ventral surface of the head (*S.O.*). The first 10 body

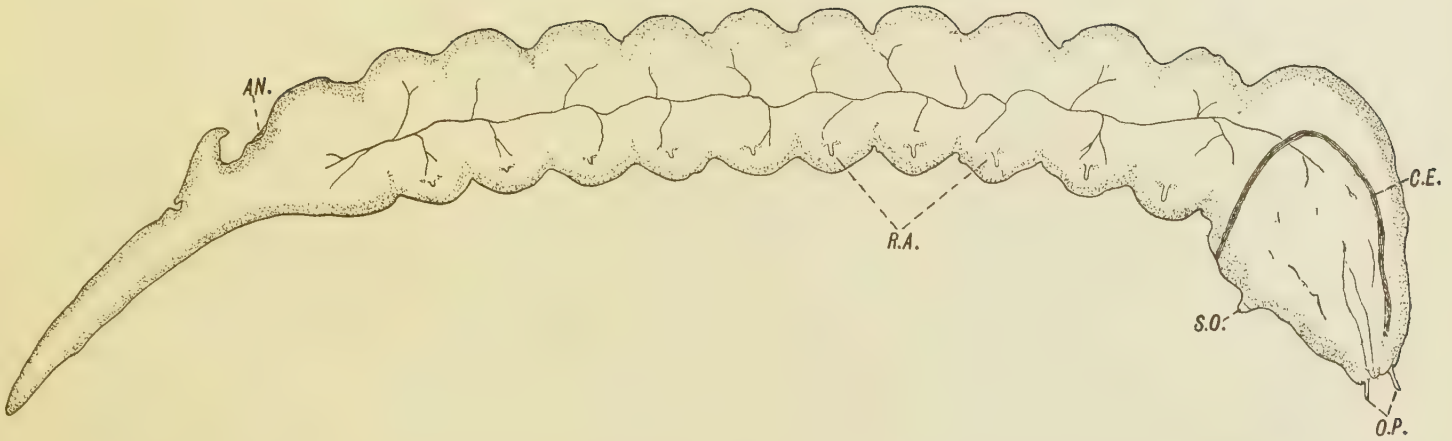

Fig. 11. The polypodeiform larva of *K. marshalli*. ×200 diams. Drawn *in vitro* with camera lucida. *AN.*, anus; *C.E.*, chitinous endoskeleton of the head *O.P.*, oral papillae; *R.A.*, rudimentary appendages; *S.O.*, sense organ.

segments each possess a pair of processes which gradually diminish in size from before backwards as in the case of the corresponding stage of *F. anthomyiarum* (Fig. 11, *R.A.*).

An apneustic tracheal system shows clearly through the transparent integument and extends through all the segments of the body.

The polypodeiform stage in neither of the Kleidotoma species here investigated differed from each other in any important particular.

The second instar lasts about 12 days and is succeeded by stages with a reduced cauda and in which the pairs of segmental processes have disappeared. The tracheal system also becomes peripneustic.

There are two broods in the case of both species during the season. Their life cycles are approximately equal in length and take from 10 to 20 days longer than *Figites anthomyiarum* Bouché.

The Control Value of Cynipid Parasites of Carrion Feeding Dipterous Larvae.

Cynipids of the sub-families Figitinae and Eucoelinae appear in considerable numbers and variety of species during the early stages of putrefaction. This is at a time when the first batch of dipterous eggs are hatching out and the parasites are thus in readiness to oviposit in the newly hatched larvae. Because of their minuteness and retiring habits they are apt to be overlooked, but their value in assisting to control undesirable Diptera which develop in putrefying media is undoubted. Among the latter may be specially mentioned the Blow Fly (*C. erythocephala*) and the sheep maggot fly (*Lucilia sericata*).

In discussing the incidence of parasitisation of the Cynipid parasites of carrion feeding Diptera a point which should be specially borne in mind is the heavy mortality which occurs among the parasitised larvae. Under both natural and laboratory conditions approximately 50 per cent. of the parasitised host larvae died. Although the parasitic larva always succumbs with the host the general effectiveness of the parasite is maintained by the high fecundity of the adult female parasite. It is perhaps worthy of note that the puparia of parasitised hosts are always below the normal size for the particular species concerned.

Parasitisation in such an early stage as the first larval instar places a severe strain on the developing host organism. It is not too much to assert that Cynipid parasites of various species are responsible for a 30 per cent. diminution in the number of Diptera developing in putrefying media. As the putrefactive processes advance and a large proportion

of the maggots reach the full grown stage the Cynipids diminish greatly in numbers and are supplanted by parasites of other groups.

A notable one is the Braconid *Alysia manducator* Panz which is capable of ovipositing in full grown maggots. Parasites of this type are succeeded in their turn by insects which parasitise the pupal stage. One of the best known pupal parasites of Diptera is the Chalcid (*Nasonia brevicornis* Ashm.).

In this way the various entomophagous parasites levy their toll at all stages in the life cycle of the host.

Discussion on the Early Larval Forms of Parasitic Cynipids.

The early stage larvae of parasitic Cynipids have a special interest, not only on account of their hypermetamorphic character, but also because some of them bear a modified resemblance to certain developmental phases which in other insects are usually passed through in the egg stage. Owing to the small quantity of yolk which the eggs of Cynipids (in common with the eggs of many Hymenoptera Parasitica) contain, and also probably because of the highly favourable conditions in which the egg is placed at oviposition eclosion from the egg is considerably hastened. Hence many of the early instars in the development of Cynipid larvae still retain resemblances to certain early embryonic forms from which they have doubtless been derived in spite of modifications due to their active intrahaemocoelic life.

Berlese(3) considers there are three distinct phases in the embryology of insects based chiefly on the stage of segmentation reached and the development of the appendages. These three stages he called successively the protopod, polypod and oligopod stages.

The distinguishing features of the protopod stage (Fig. 12 *A*) are the incomplete state of the abdominal segmentation and the fact that pairs of appendages are confined to the cephalic and thoracic segments. The internal organs are very rudimentary and there are as yet no indications of circulatory or tracheal respiratory systems.

In the polypod stage (Fig. 12 *B*) segmentation is complete, all the segments have acquired appendages and there is evidence of a tracheal respiratory system.

In the oligopod stage (Fig. 12 *C*) the abdominal appendages have been resorbed whilst those of the thoracic segments have increased in size.

As Imms(11) points out in his admirable summary on types of insect larvae, the latter represent arrestations in one or other of the above embryonic phases when eclosion from the egg takes place.

Our present knowledge of the primary larvae of Cynipoidea is confined to six species, all of which belong to the parasitic forms. Keilin and Pluvinel(12) have described the primary larva of *Eucoila keilini* (Kieff.) an endoparasite of *Pegomyia winthemi* (Mg.) in the latter's larval stage. Haviland(10) has given an account of the first stage larva of *Charips* (*Allotria*), a hyperparasite of aphids through *Aphidius* (Braconidae).

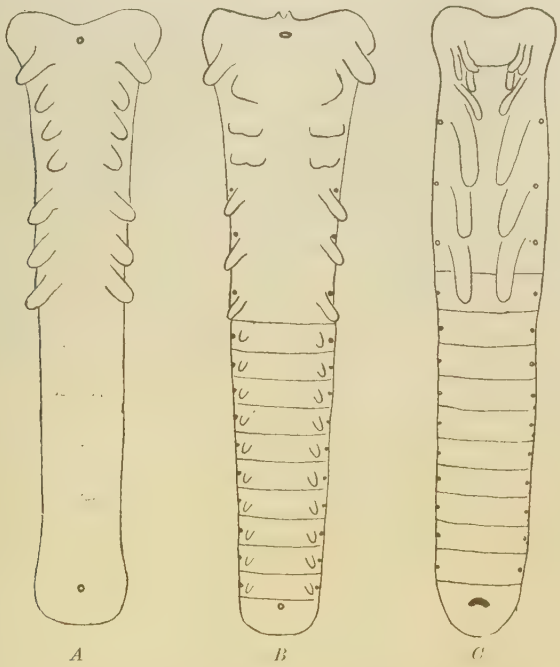

Fig. 12. Three embryonic phases of insects. From Imms, *Textbook of Entomology*. After Berlese. *A*, protopod; *B*, polypod; *C*, oligopod.

These latter, with the primary larvae described in this paper, bring the number to six.

An examination of the known primary larvae of species taken from the genera *Cothonaspis* (Hartig), *Eucoila* (Westel), *Kleidotoma* (Westd.) all belonging to the sub-family Eucoilinae, suggest that most of the species of the sub-family possess primary larvae varying little from the characteristic eucoiliform type.

The primary larvae of *C. rapae* is remarkable for the extraordinary development of the thoracic processes which are proportionately longer than those of *Eucoila keilini* (Kieff). Undoubtedly eclosion from the egg in these two forms occurs in the middle of the protopod stage. The two primary larvae of Kleidotoma already described in this paper possess relatively much shorter thoracic processes than those of *Cothonaspis rapae* or *Eucoila keilini*, although exhibiting all the other primitive characters associated with the protopod stage. It is difficult to decide which pair represents the earlier ontogenetical stage.

The primary larva of *K. marshalli* with its better developed segmentation probably hatches at a later embryonic stage than the other three first stage eucoiline larvae here discussed.

The structure of the anus in all the eucoiliform larvae I have seen bears affinities to the enlarged anus described by Haviland (10) for the primary larvae of *Charips*.

The primary larva of *Figites anthomyiarum* Bouché described in this paper is the only one about which anything is known in the quite considerable sub-family of the Figitinae. The first stage larva of *F. anthomyiarum* is obviously a modified eucoiliform and its well-defined segmentation places it as hatching later in embryonic development than any cynipid primary larva yet described. The first stage larva of *Charips* (*Allotria*) differs from all other Cynipid primary larvae yet examined in that it is devoid of thoracic processes.

Very reduced thoracic processes appear in the second instar. As the second stage larva of *Charips* approximates closely in essentials to a protopod larva, it seems reasonable to regard the primary larva as representing something in ontogeny prior to or at the beginning of the protopod stage.

From this examination of the known primary larvae of Cynipoidea the weight of evidence suggests that eclosion from the egg in at least the parasitic members of this group takes place at or near the protopod stage. This conclusion is further strengthened by the fact that the writer has succeeded in demonstrating in at least three cases that the primary larva is followed by a form with definite polypod characteristics.

As already described, the primary larvae of *F. anthomyiarum* and the two Kleidotomids were succeeded by an elongated larva possessing a rudimentary pair of appendages on each of the first 10 body segments. Although still very simple the alimentary canal and nervous system are better organised than in the protopod stage and an apneustic tracheal system has developed.

There can be little doubt that this instar represents the polypod embryonic stage of Berlese(3). The writer is unaware of any described type of larva among the endoparasitic Hymenoptera which exhibits polypod affinities so unmistakably. This type of larva is therefore termed the polypodeiform larva. The migratory planidium larva (Chalcidoidea) described by Smith(14) and Timberlake(19) may be another modification of the polypod stage. The pairs of spine-like locomotory processes on each body segment except the last one may conceivably represent modified appendages. The writer is also of the opinion that a great number of the parasitic Cynipids which possess a definite protopod type of primary larvae will be found to have polypod stages in addition. The question naturally arises, is there a larval instar in Cynipoid development which has definite oligopod affinities? The mode of life of these endoparasitic types makes it impossible to follow throughout the development of any individual parasite and reliance must of necessity be placed on a large number of time stage dissections. Consequently one would not care to be too confident that an oligopod stage will not yet be found, possibly of a very transient nature, even in the species studied in this paper.

Experiments bearing on Host Selection among Cynipid Parasites.

The freedom with which Cynipid parasites will oviposit in carrion feeding dipterous maggots irrespective of species has already been alluded to, but this facility was not found to extend to phytophagous dipterous maggots.

Thus, although *F. anthomyiarum* will oviposit in many species of young saprophagous Anthomyids it refused to oviposit in the young larva of a phytophagous Anthomyid, *Hylemyia brassicae*. As the young larvae of *H. brassicae* are approximately the same size, shape and colour as its usual hosts it was presumed that odour was the repellent factor. Therefore the following experiment was conducted. A small number of first instar larvae of the species *H. brassicae* were thoroughly washed in water to remove the strong odour of cabbage plant sap and were then placed in a small quantity of very putrid meat which had just previously been the nidus of carrion feeding maggots. The *H. brassicae* larva by their constant wriggling soon became coated with the juices of the meat. The whole was then transferred to a breeding tube and several lively young females of *F. anthomyiarum* introduced. Oviposition actually

took place as was proved by subsequent dissection of some of the maggots and discovery of the parasite's eggs.

The remaining larvae were restored to their natural pabulum and only a small percentage died. The remainder developed into healthy full grown maggots without exhibiting any trace of parasitism. Later dissections revealed parasite eggs in various stages of degeneration in the larval haemocoels.

The conclusion appears to be that the odour of carrion stimulates the female parasite to oviposit, but there is some condition in the haemocoelic fluid of a phytophagous maggot which inhibits the development of a parasite accustomed to develop in the haemocoel of a saprophagous maggot.

An attempt was made to induce *C. rapae* to oviposit in young carrion feeding anthomyid larvae after the latter had been well washed and smeared with the expressed juice of cabbage roots. Owing probably to being unable to faithfully reproduce the conditions necessary for oviposition the latter did not take place as no eggs were found on subsequent dissection of the larvae.

Summary.

(1) *Cothonaspis rapae* (Westd.) was found to be an effective parasite of the Cabbage Root Maggot (*Hylemyia brassicae* Bouché) in the Cambridge district. Twenty-five per cent. of the total of host maggots and puparia examined were found to be parasitised by this cynipid.

(2) The average duration of a life cycle of *C. rapae* is 92 days and the length of the larval stage is about 55 days. There are two generations in the season; the first appears in May and the second in August and September.

The winter is passed in the larval stage.

(3) Hypermetamorphosis occurs in the life cycle, the primary larva being eucoiliform with three long pairs of thoracic processes, a long cauda, and suctorial mouth parts. The full grown larva is a typical mandibulate hymenopterous grub without cauda or appendages of any kind.

(4) The life-histories of *Figites anthomyiarum* Bouché, *Kleidotoma marshalli* (Marshall) and an undetermined *Kleidotoma* are described.

They are all effective parasites during the early larval stages of carrion feeding Diptera such as the *Calliphora erythrocephala*, *Sarcophaga carnaria*, *Lucilia sericata*, etc. The writer estimates that saprophagous

maggots are reduced in numbers by 30 per cent. owing to parasitisation by various Cynipid parasites during the early larval stages.

(5) These three parasites have each two broods per season. The duration of the Figitids' life cycle is about 60 days, but that of both Kleidotomids takes from 10 to 20 days longer. There does not appear to be any definite period of emergence, but one generation overlaps another.

(6) The primary larva of *F. anthomyiarum* is a modified eucoiliform. No primary larva of a figitine species has been hitherto described. The primary larvae of the two Kleidotomids are eucoiliform but with short thoracic processes. These types of primary larvae are each succeeded by an entirely new type of endoparasitic larva which bears pronounced affinities to the polypod embryonic stage. The writer has termed this type of larva the polypodeiform larva. The polypodeiform larva of both *F. anthomyiarum* and *Kleidotoma marshalli* are figured and described.

The early stage forms of parasitic Cynipid larvae are reviewed and compared.

(7) Several experiments were conducted to test whether Cynipids parasitising saprophagous maggots would oviposit in phytophagous maggots and *vice versa*. Under suitable conditions *F. anthomyiarum* was induced to oviposit in the maggots of *H. brassicae* (phytophagous) but the eggs did not develop.

C. rapae, however, could not be induced to oviposit in saprophagous Anthomyid larvae.

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REVIEW

The Potato: its History, Varieties, Culture and Diseases. By THOMAS P. MCINTOSH. Pp. xvi + 264; 38 figs. Oliver and Boyd, Edinburgh. 1927.

The potato is one of the two important foodstuffs in regard to which Great Britain is still practically self supporting. The annual value of the crop in this country is computed to be about £30,000,000, but in addition it has fundamental importance in that it retains more labour on the land than probably any other of our farm crops. On the other hand it is very subject to disease; more capital is involved per acre and cropping and price fluctuate more than those of any other common crop. In the present precarious state of our agriculture it is essential that all knowledge concerning so primary a crop should be as widespread and easily available as possible and a readable and up-to-date treatise on the potato is therefore very welcome.

There have, of course, been the valuable German works by Snell and American books by Grubb and Guildford, Gilbert and Stuart, but apart from Findlay's volume of 1905 there has, until recently, been no English book on the subject. The gap was partially filled by Salaman's work, published in 1926, but this author did not attempt to cover the whole field and his volume is much more a technical source book for research workers than a general account of the potato. The present volume by McIntosh is a general account and more resembles Stuart's book.

An interesting historical introduction giving an account of the origin and development of the potato with personal notes on those who have played a prominent part in the breeding of new varieties is followed by seven chapters on the botanical aspects of the subject, more particularly dealing with problems of varietal classification and the maintenance of pure stocks. Five chapters are then given to questions of potato breeding and propagation, quality and productivity, and three chapters to cultivation, manuring and utilisation. The next sixteen chapters, some very short and one containing only ten lines, are devoted to various aspects of disease in potatoes. Appended are descriptive notes on 39 common varieties, a glossary and a somewhat incomplete index. There is a prefatory note by Prof. J. A. S. Watson, of Oxford, and the book is illustrated by 38 figures, not many of which are original.

The author intentionally omits any discussion of marketing and synonymous nomenclature, but for these refers interested readers to easily accessible and recent official publications in which they are dealt with at some length.

The volume is very unequal, the author who is an Inspector to the Board of Agriculture for Scotland being, as one might expect, at his best when dealing with problems of varietal classification and the field aspect of the crop. Part V, however, which is a quarter of the book, and deals with "Diseases, pests and injury" is not good except again when the author is dealing with the field aspects. Some of these chapters give the impression of having been inserted hurriedly in the proof sheets, and if a second edition is called for it is to be hoped that this section will be considerably improved.

The volume will be found very useful in that it assembles in convenient and systematic form a great amount of information regarding the potato that otherwise is scattered in journals which are often not readily accessible. It certainly meets a need and will be welcomed not only by teachers and students of agriculture but by all practical men dealing with the potato crop.

WILLIAM B. BRIERLEY.

PROCEEDINGS OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS

ANNUAL GENERAL MEETING held at 5 p.m. on Friday, January 20th, 1928, at the Imperial College of Science and Technology, London.

Address delivered by the retiring President, Mr J. C. F. FRYER, M.A., on "Legislation in England against Diseases and Pests of Plants."

WHEN seeking for a subject for my address, I at first proposed to discuss in general terms plant quarantines and the attempts which all civilised countries now make to exclude from entry insect, fungus, and other plant pests. I was, however, reminded that as there is no discussion on the President's address, my first proposal would prevent the Association as a whole from dealing with a topic both interesting and controversial, and it was suggested that I should instead confine myself to the development of English legislation against insects, fungi, etc., leaving my original subject free to occupy some subsequent meeting. I am adopting this suggestion, and offer this explanation as to why I have chosen what I fear is a somewhat dry aspect of an interesting subject.

The Destructive Insects Act, 1877

In the first place, and I think this applies to most countries, it must be recognised that the earliest efforts to deal with pests by legislation were not based upon any comprehensive examination of the whole subject, but were rather dictated by isolated events which brought about results sufficiently serious and startling to create something like a panic in important sections of the general public. So far as England was concerned, the first event to bring about legislation was the remarkable spread of the Colorado Beetle across the continent of America between the years 1859 and 1874, the discovery of isolated beetles on ships arriving from America in 1876 and 1877, and the attempt at colonisation made by the beetle at Mülheim in Germany in the latter year. At that time the disastrous effects of the introduction of Potato Blight were still relatively fresh in the memory of the public, and the apprehension of what might occur should the Colorado Beetle be introduced persuaded Parliament in 1877 to pass an Act, which, though entitled "An Act for Preventing the Introduction and Spreading of Insects Destructive to Crops," actually only dealt with the Colorado Beetle. At this time there was no Board of Agriculture, and the responsibility for administering the Act was confided to the Privy Council. The powers given were very wide, and enabled the Privy Council to prohibit or regulate the landing of potatoes, potato haulm, or any other substance or article which might be likely to introduce Colorado Beetles. It also enabled the Privy Council to secure the destruction of any crop or substance on which the insect was found or to which it appeared likely to spread, and finally to prohibit the introduction, keeping, or sale of living Colorado Beetles. I don't want to trouble the Association with details of administration, but it is important to note that where the Privy Council directed

the removal or destruction of a crop, they were allowed to instruct the Local Authority of the district concerned to pay out of the rates compensation on a scale laid down in the Act. It was evidently realised at the time that the safest method of dealing with an outbreak in its early stages is to destroy the affected plants, and that such destruction, which is in the interests of the community at large, could not be affected without compensating the owner of the plants.

The Privy Council took advantage of this Act and passed certain Orders against the Colorado Beetle, but it was not until August 1901, by which time the powers of the Privy Council had been transferred to the Board of Agriculture, that it became necessary to cope with an outbreak—at Tilbury—when the powers under the Act appear to have been adequate to the task, since by the end of 1902 the pest was eradicated. One year before this outbreak, however, in 1900, occurred the second event which was ultimately to bring about changes in the laws of the land –the appearance of American Gooseberry Mildew in Ireland. During the following five years, a valued member of this Association, Prof. Salmon, drew attention repeatedly to the dangers of this new disease, and in 1906 his fears were fulfilled, for during that year and the one following, American Gooseberry Mildew was not only found in England, but found to be already strongly established. Public opinion again made itself felt, too late unfortunately so far as American Gooseberry Mildew was concerned, and in 1907 an Act was passed to amend the Destructive Insects Act of 1877 so as to allow the inclusion, in addition to the Colorado Beetle, of "all such insects, fungi, and other pests" as were "destructive to agricultural crops or to trees or bushes."

The Destructive Insects and Pests Act, 1907

Two points in connection with this Act are of importance. In the first place, the Board's powers in regard to compensation for destruction were greatly curtailed, in that such compensation could only be paid if the Local Authority agreed to provide the necessary funds. In the earlier Act the Local Authority had no choice in the matter, since if instructed to pay it had to find the funds, apparently regardless of the amount in question. This curtailment of the Board's powers was perhaps not unnatural, and specially at the time when the Act was passed, because the Board was then being pressed to cut down and burn all gooseberry plantations affected by American Gooseberry Mildew, a policy which, if followed, would have involved, in some counties, heavy burdens on the rates. Nevertheless, it was a retrograde step, which might have turned the scale in favour of some foreign invader.

The second important point in the amending Act was the wording of the sentence by which pests other than the Colorado Beetle were brought within the scope of legislation. When those drafting the amending Act used the phrase "any insect, fungus or other pest" they probably imagined they were using a comprehensive expression enabling the Board to deal with every sort of plant pest and disease, but it was held subsequently that, by a well-established legal usage, the term "other pests" was limited by the earlier words "insects and fungi" to pests of an insect or fungus nature. It was therefore at least questionable whether under this Act the Board, and subsequently the Ministry, have had powers to deal with bacteria or nematode worms—still less with "virus" diseases, which were not, I think, recognised as such at the time when the Act was passed.

The Acts of 1877 and 1907, however, served their purpose for a period of 20 years, and under them has been evolved the machinery now in force. This evolution has

been a gradual process, and it is of some interest as it is more or less typical of what has happened in other countries as well as in England.

It has been explained that both Acts were directed essentially against the menace from abroad, which menace was felt in the minds of those concerned with the matter as being due to a somewhat limited number of notorious pests rather than to a whole host of unknown but potentially dangerous organisms. Almost before the passing of the 1907 Act, however, changes in the situation were taking place, which ultimately caused considerable modification in the attitude of those concerned with anti-pest legislation. In the first place, by the time operations under the 1907 Act had begun, American Gooseberry Mildew had obtained such a firm hold on the country that it was not—and, indeed, probably could not have been, eradicated. Therefore legislation brought into being on account of the menace from overseas was actually being used to deal with a pest which, though of foreign origin, had nevertheless become to all intents and purposes an established resident. This initial and small extension of the objects underlying the Acts of 1877 and 1907 was further developed when it became necessary in 1908 to deal administratively with Wart Disease of potatoes, for whatever the origin of this disease may have been, there is no doubt that long before 1908 it had obtained so firm a hold on the country that it could only be regarded as a resident.

From 1907, therefore, proceedings under the Destructive Insects and Pests Acts tended to diverge into two separate channels. There was first the action taken to prevent foreign pests from entering the country, and secondly that to deal with pests already established and resident (although many, perhaps most, of them have at some time been introduced from abroad.

Legislation in regard to Foreign pests Taking first the legislation aimed at the foreign pest menace, the initial Order under the Act of 1907 was an Order issued in June 1908. All it did was to require the notification to the Board of the existence of certain scheduled pests, and to render it illegal to keep or sell living specimens of them. Nothing was said as to prohibiting the importation of scheduled pests, neither were powers taken to deal with them when found, and the Order is chiefly of interest as showing that the dominant idea at the time was to proclaim or outlaw notorious pests without committing the authorities to any special line of action.

The Order of 1908 was followed by an Order of 1910 which scheduled the following foreign insects and diseases not known to occur in Britain: Phylloxera of the Vine, San José Scale, Mediterranean Fruit Fly, Colorado Beetle, Potato Moth, Cherry Fruit Fly, Black Knot (*Plowrightia morbosa*) and Pear or Fire Blight. Certain other pests were also scheduled, but they were either resident or quasi-resident, and must be dealt with later. The essential requirements of the Order of 1910 were first that the scheduled pests must not be imported, secondly that the occupier of any premises on which a scheduled pest existed must notify the Local Authority or the Board, and thirdly that he must carry out such measures for dealing with an outbreak as the Board required, except that he could not be compelled to destroy a plant or crop unless the Local Authority had agreed to pay compensation. This method of dealing with the danger shows a distinct advance on the Order of 1908, but it obviously depended for its effectiveness first on the ability of the legislator to select the right pests for his schedule, and secondly on that of the occupier or plant

importer to recognise a scheduled pest when he saw one. As regards the latter condition, compulsory notification was probably copied from the Diseases of Animals legislation, but it was perhaps not realised that whereas practically every farmer called in the veterinary surgeon to a sick animal, he did not (at that time at all events) often seek for a plant doctor when his crops were in trouble: in consequence, compulsory notification was only effective in the case of such pests and diseases as the importer or occupier could not fail to recognise. For this reason it is doubtful whether the Order, which operated for 11 years, did anything whatever to prevent the introduction of foreign pests, though where by chance or good management such a pest was discovered after arrival, it enabled the Board to take action—as, in fact, occurred in one or two instances.

These remarks may seem to imply some criticism of those responsible for preparing and administering the Acts and Orders in the pre-war period, but a brief reference to the legislation in force during that period in one or two other countries will show that no criticism would be warranted. Taking first the United States as the country which has since paid most attention to plant import regulations, it will be found that in 1912 there were no Federal laws on the subject, and as regards the administration of the State laws, the Bureau of Plant Industry (*Bulletin* 206) wrote as follows: "The States vary much in the efficiency of their inspection laws and in the execution of those laws. Even in the best protected States it is not uncommon to find lots of stock which have gotten into the State without the inspector having been informed. In the other States it is very common for such lots to gain entry entirely unknown to the proper authorities. Besides all this, the State laws apply only to stock raised within the State or shipped to some point within and then unpacked or planted out. That is, any amount of stock may be imported and shipped again to other States without being required to pass any inspection at all."

As regards Europe, we find that in most cases plant import regulations were governed by the Phylloxera Convention of 1881, which was aimed at preventing the spread of Phylloxera and not other pests. Some of the overseas Dominions and Colonies of the Empire, to their credit, already had legislation far more in accordance with modern opinions than Britain, but as a whole the system in force in the latter country in 1910 compared not unfavourably with those current in Europe and much of the rest of the world. The fact that that system now appears to be ineffective is not criticism of those responsible for it but is rather an indication of the development of knowledge and method which has subsequently taken place.

As a matter of fact, the inherent weakness in the mode of procedure under the Order of 1910 was recognised within a very short time, but revision was deferred first owing to an attempt made at a Conference in Rome in 1914 to persuade all countries to adopt similar methods in dealing with the foreign pest menace, but subsequently and chiefly by the Great War. In 1921 revision was at last possible, and it became necessary to decide what system should be adopted. Since 1907 the ideas of the world on the subject of plant import regulations and quarantines had advanced greatly, and had crystallised in three different forms. There was first the school which favoured the complete embargo upon trade in all plants likely to carry pests; secondly that which favoured the introduction of plants from abroad only if they had been subjected to disinfection; and lastly that which relied upon some process of inspection, to be carried out either in the country of origin and to be

vouched for by a certificate of health, or in the importing country when plant consignments passed the Customs. At this point it is not proposed to discuss the merits or demerits of these different systems, and it will be sufficient to say that England, in Orders of 1921 and 1922, adopted the certificate system, which remains in force at the present time.

It is not necessary to trouble you with the details of these Orders, but the essentials are as follows: the categories of plants regarded as dangerous owing to their ability to carry foreign pests are scheduled, and they can only be imported if they have received a certificate of health from the exporting country, or failing that have been examined and released by the Ministry of Agriculture. The most recent Order follows its predecessors in containing a schedule of foreign pests, also two or three residents, and the certificate claims that the plants covered by it are healthy and free from scheduled pests. Powers are taken to deal with scheduled pests and plants affected by them, while the importation or sale of living specimens of such pests is also illegal.

The pests scheduled in the Order are as follows:

Fungi. Black Knot of Plum and Cherry (*Plowrightia morbosa* Sacc.).
Fire or Pear Blight (*Bacillus amylovorus* Trev.).
Chestnut Canker (*Endothia parasitica* (Murr.) Ander. and Ander.).
Wart Disease or Black Scab of Potatoes (*Synchytrium endobioticum* Perc.).
Onion and Leek Smut (*Urocystis cepulae* Frost).
Downy Mildew of Hops (*Peronoplasmopara humuli* Miy. et Taka.).

Insects. Vine Louse (*Phylloxera vastatrix* Planch.).
American Apple Capsids (*Heterocordylus malinus* Reut. and *Lygidea mendax* Reut.).
Pear Tingid (*Stephanitis pyri* Fab.).
Colorado Beetle (*Leptinotarsa decemlineata* Say.).
Plum Curculio (*Conotrachelus nenuphar* Herbst.).
Potato Moth (*Phthorimaea operculella* Zell.).
American Lackey Moths (*Malacosoma americana* Fab. and *M. disstria* Hubn.).
Oriental Fruit Moth (*Cydia molesta* Busck.).
San José Scale (*Aspidiotus perniciosus* Comst.).
Japanese Fruit Scale (*Diaspis pentagona?* Newst.).
Apple Fruit Fly (*Rhagoletis pomonella* Welsh).
Cherry Fruit Flies (*Rhagoletis cerasi* Linn., *R. cingulata* Loew. and *R. fausta* Osten Saken).
Gooseberry Fruit Fly (*Epochra canadensis* Loew.).

Legislation and the Resident pest It is hoped in conclusion to refer again to this Order, but before going too far ahead we must retrace our steps and follow the evolution of the legislation dealing with the resident pest.

First, reference may be made to some of the pests of this character which appeared in the schedules of the 1910 Order—the Large Larch Sawfly and the Nun Moth being good instances. As regards the Large Larch Sawfly, the insect had just begun to do very serious damage in the Lake District and fears were expressed that the larch was doomed. It was considered that it might be necessary to deal with the pest by some such administration as was in force in connection with American

Gooseberry Mildew: therefore, as a preliminary step it was scheduled in order to secure compulsory notification and so obtain knowledge as to the distribution of the species. Somewhat similar reasons were probably responsible for scheduling *Septoria lycopersici* and *Mycosphaerella citrullina*. As regards the Nun Moth, an insect widely resident in the South and Midlands of England, the reason for scheduling was quite different. The insect had never been known to cause harm in England, and in any case it was not amenable to administrative action in the woods in which it occurred. At the time, however, foreign countries, and notably the United States, were steadily tightening their import regulations, and this latter country, suffering very seriously from the Gipsy Moth, was not unlikely to look with equal fear at the Nun Moth, a serious pest on the Continent of Europe. The insect was therefore scheduled in the interests of those nurseries which had then a valuable export trade to the United States, since it was felt that this action would at least ensure that such nurseries should be free from the insect. The same idea was responsible for scheduling the Gipsy Moth and the Narcissus Fly, the former an insect which had become extinct in Britain in spite of repeated efforts to reintroduce it, and the latter already widely distributed but causing possible risks to the bulb trade with New Zealand. The scheduling of such pests as these in the 1910 Order, however, never led to any serious efforts to control the pests in question, and the action must be regarded chiefly as an attempt to discover to what use the Act of 1907—then a new and unfamiliar weapon—could be put. Far more important was the action taken against American Gooseberry Mildew and Wart Disease, in connection with which a large number of Orders were issued.

American Gooseberry Mildew. It is impossible to give an adequate summary of the action taken against this disease, but some effort to trace the principles underlying such action and the reasons for its ultimate failure are desirable. As soon as the Board had powers under the Act of 1907, it followed an eradication policy, attempting to have all diseased bushes burned. This attempt at once showed the disease to be relatively so widely spread that measures of eradication were impracticable. The next series of Orders therefore endeavoured to secure control of the disease on affected premises, and prevent its spread to those adjacent but unaffected. Therefore at one or other time growers were compelled to spray, to cut off and burn the disease in its winter stage on the twigs, and to burn diseased fruit, while the distribution of bushes from affected premises was prevented. This, again, proved a failure, partly on account of the nature of the disease, which was so easily spread, partly because the research necessary to justify spraying had not then been carried out, and partly because the mechanical measures were not very effective in practice even if they could have been completely enforced—which was not the case. The disease therefore continued its spread. The next series of Orders differed little in the precise measures they enforced but instead of treating infected orchards as separate units, large areas were scheduled as infected, with the object, if possible, of limiting the disease to such areas. The same reasons which rendered the measures ineffective when applied to single orchards made them equally ineffective as regards large areas and the distribution of the disease became practically universal in that all the chief gooseberry growing districts of the country were declared "infected areas." A penultimate series of Orders, issued from 1915 onwards, required all growers in such areas to burn all the diseased berries and all diseased

wood before September 30th and rendered the sale of diseased berries illegal, as also the movement of bushes out of an infected area without a licence. Since practically the whole country was then infected, these measures were perhaps intended rather to prevent avoidable loss in gooseberries than the spread of the disease. At all events, the results obtained were very dubious, and from 1919 onwards the attempt to control American Gooseberry Mildew was largely abandoned. At the present day the only remaining restrictions are, first that bushes substantially affected must not be sold, and secondly the requirements of a health certificate with imported berries. The attempt to control American Gooseberry Mildew by administrative measures thus ended in failure, which was probably inevitable from the start. It had, however, at least the value of demonstrating certain truths, the most important being that if an invading disease is to be eradicated, it must be dealt with in the very earliest stages of the outbreak.

Wart Disease. The history of the Wart Disease administration offers some parallels to that concerned with American Gooseberry Mildew, and it might have had a similar ending if it had not been for two very important distinctions between the two diseases. In the first place Wart Disease, essentially a soil disease, is far less rapidly spread than American Gooseberry Mildew, so that more time was available to develop administrative methods of control; and secondly, varieties of potato immune to the disease were soon found to exist. As in the case of the mildew, the first efforts made by the Board were designed to destroy and prevent the appearance of disease on infected premises by burning diseased material, by dressing the soil, and by preventing the distribution of diseased tubers. The inspection necessary in connection with these measures showed the disease to be much more widely spread than was at first supposed, and as in the case of American Gooseberry Mildew, infected areas were substituted for infected premises. The first real step in the control of the disease was, however, Mr G. C. Gough's observation that certain varieties were immune, and subsequently the late Mr Snell's Ormskirk trials, which showed conclusively which varieties remained immune under conditions most conducive to infection. The Board was thus provided with a thoroughly effective means of controlling the appearance of Wart Disease such as was never the case with the Gooseberry Mildew, and the planting of immune varieties of potatoes on infected land was therefore enforced. As a natural consequence of this action, it became necessary to enable growers to obtain immune varieties, and specially such varieties free from susceptible "rogues," whence, as a part of the administration of the Wart Disease Orders, the supervision of the growing of seed potatoes of immune varieties was evolved.

The enforced planting of immunes did not, however, sufficiently prevent the continued spread of the disease to new territories, and in consequence a revised policy was adopted, which was in reality the result of a fresh outlook on the whole problem. Up to this time attention had chiefly been directed to the infected areas, which had perhaps been visualised mentally as black patches in an otherwise clean country. Now, however, this outlook was reversed and the clean areas were regarded as white patches in an otherwise black country, and instead of attention being concentrated on the infected areas, it was transferred to those still clean. As a result, the country was boldly divided into two divisions, one largely infected, and one clean, and steps were taken to ensure not only that the infected areas should be

supplied with immune seed but that the clean areas should get seed free from infection. With this object in view it was decided to require that no potatoes should be planted or sold for planting unless they had been certified to be either approved immune varieties or to have been grown on land free from Wart Disease, while no potatoes should be moved out of the infected area except ware of immune varieties. This policy has, in point of fact, resulted in a most marked reduction in the rate of spread of Wart Disease. More than this cannot, of course, be expected, but the delay in the rate of spread is itself worth while, for not only is the potato breeder given time to add to the list of immunes, but large potato growing areas are enabled to retain a valuable export trade which would otherwise be lost, owing to the fear of foreign countries of introducing the disease.

Other resident diseases. A third disease now the subject of administrative action is Onion Smut, which, however, owing to the lack of time must be very briefly dismissed. It will be sufficient to say that the disease is of very restricted distribution, and that the action taken is largely based upon the fact that onion plants are not susceptible after they have passed the seedling stage. Occupiers of land infected by Onion Smut are therefore only allowed to grow onions and leeks in accordance with the conditions of a licence.

These three series of Orders were all directed against serious resident diseases of local distribution (or at all events originally of such distribution). In a somewhat different category is the action taken against Silver Leaf, a fungus disease which between 1910 and 1920 became more and more prevalent in every plum growing district in the country. Here it was not a case of a pathogenic organism spreading over and conquering new territory, but rather of the disease becoming more virulent and doing more damage.

The disease, therefore, differed fundamentally from those which had previously been brought within the scope of the D.I.P. Orders, and marked a further stage in their evolution. The reasons for dealing with Silver Leaf were threefold. The first, and least important, was of a temporary character. During the War measures of good cultivation were imposed upon growers, and for a time afterwards a similar attitude towards agriculture and horticulture persisted and encouraged measures of compulsion in the case of such a disease as Silver Leaf. A second, and more persistent, reason for dealing with the disease was that the negligent grower by refusing to take reasonable precautions may infect not only his own trees but those of his neighbour. This, of course, applies to many pests besides Silver Leaf, but the singling out of the latter disease is explained by its deadliness—which provides the final reason for administrative action. As the Silver Leaf fungus only fructifies on dead wood, the Order merely requires plum (and apple) growers to cut out and burn all dead wood before a certain date—at first April, but now, upon the advice of Mr Brooks, the 15th July. Inspectors also have power to compel the destruction of any dead wood of any kind upon which the fruiting stage of *Stereum purpureum* is found.

We have now passed from the attempts to deal with an introduced but established fungus such as American Gooseberry Mildew to those made to control a serious indigenous disease such as Silver Leaf. From the latter it is but a short step to the last series of Orders at present in operation—the Sales of Diseased Plants Orders. When legislation against resident pests was first put into force, it was intensely unpopular, but with growing experience on the part both of the authorities

and of farmers and fruit growers, a more tolerant attitude developed and received a further impetus from the co-operation between authorities and growers brought into being as a result of the War. A further result of the War was a marked shortage of nursery stock, and in consequence the sale of much diseased and unhealthy stock which, under other conditions, would have been destroyed. There occurred therefore simultaneously an obvious evil with which the Destructive Insects and Pests Acts were capable of dealing and a new readiness on the part of growers to make use of these Acts, with the result that an Order was issued rendering it illegal to sell plants substantially attacked by certain common and generally distributed pests. Complete freedom from such pests could not reasonably be asked, but the purchaser of nursery stock could clearly demand first that the plants should not be so damaged as to be incapable of growing satisfactorily, and secondly that they should not be infested to such an extent as to render them a menace to any surrounding healthy trees among which they might be planted. This Order—the Sale of Diseased Plants Order—presents certain administrative problems with which there is not time to deal, but it may be worth mentioning that for the first time pests and diseases are scheduled by groups—*e.g.* all scale insects and all organisms responsible for fruit tree cankers.

Destructive Insects and Pests Act, 1927 We now come to the most recent development of the Destructive Insects and Pests Acts, which took place as lately as last autumn. It has already been mentioned that it was doubtful whether, under the Act of 1907, the Board—and subsequently the Ministry—have had powers to deal with any plant pests other than insects and fungi. There was also a second difficulty arising out of the Act of 1907, and this was the inability of the Ministry to compensate occupiers for the compulsory destruction of plants attacked by foreign scheduled diseases unless the Local Authority had previously agreed to find the money, a proviso, which, though designed in the interests of economy, might nevertheless have exactly the opposite effect, since delay in dealing with some serious invading pest would not only involve a heavy expenditure upon administration, but might also result in the victory of the pest.

An Act has therefore been passed during the recent Session which makes it clear that the Ministry has powers to deal not only with insects and fungi but also with "bacteria and other vegetable or animal organisms, and any agent causative of a transmissible crop disease." It also authorises the Minister to pay compensation for destruction up to £2000 a year without Treasury sanction, so that immediate action can be taken in the case of outbreaks of dangerous foreign pests and diseases. Finally, the opportunity was seized of clearing up certain other points—as, for instance, the powers of inspectors to ensure the destruction of plants attacked by a foreign invader where the owners either could not or would not do so.

General Conclusion The evolution of British legislation against plant pests and diseases has now been traced up to the present day, but in such an address as this it is impossible to deal adequately with so complex a subject, and in consequence my survey has necessarily been somewhat sketchy and incomplete. In conclusion, therefore, it seems desirable to discuss certain aspects in greater detail.

As regards administration and the established pest, I have little to add to the remarks already made, because the measures which can be applied vary so greatly

in accordance with the nature of the pest and the circumstances at the time when action is taken, and in consequence generalisations are rather dangerous. It is, however, possible to detect a development of opinion in two directions: whereas in earlier years occupiers of premises infected by some scheduled (but established) pest were required to carry out measures prescribed in considerable detail, the tendency now is to allow such occupiers the greatest possible freedom as to the precise treatment they should adopt, provided always that they do not endanger their neighbours or the country at large. In other words, the less harassing the requirements can be made, the better the results are likely to be. The second development arises out of the increasing recognition of the importance of planting healthy seeds, trees, or plants, and in consequence the demand for administrative measures to eliminate, or at least prejudice, the sale of seed and plants seriously infected by diseases or pests. This demand may lead to some extent to further legislation along the lines of the Sales of Diseased Plants Order, but perhaps even more to the development of systems of certification which will be adopted voluntarily by the sections of the public concerned, the measures required being carried out under the shadow of the Destructive Insects and Pests Acts but without the necessity for taking legal powers.

Finally, as regards the menace from foreign pests and diseases: it may be recalled that the inspection or certificate system was chosen as the first line of defence. Such a system is open to the very obvious criticism that the best of inspectors, whether at home or abroad, cannot detect every pest or disease. The most that he can do is to ensure that plants passed by him are of a high standard of health, which is of course in itself a considerable gain, because the smaller the numbers of a species which are imported, the less chance there will be of that species effecting a settlement. This gain would not, however, in my personal opinion, justify the retention of the inspection system were it not possible to reinforce it in certain ways. Of these the first is the use of the embargo in cases where inspection, however carried out, could not disclose the presence of some specially dangerous or troublesome pest. There are three such embargoes in operation at the present time—the first dealing with potatoes from countries in which the Colorado Beetle exists, the second with elm trees from Europe on account of Dutch Elm Disease, and the third, which is of a partial nature, with cherries owing to Cherry Fruit Fly. The imposition of embargoes, however, must obviously be severely limited if the trade advantages of the inspection system are to be retained: therefore two further forms of reinforcement are necessary. One of these consists in an arrangement whereby consignments of plants from certain countries or continents are re-examined during their first season of growth in Britain. The countries to which this applies are those such as North America and Japan with a temperate or warm temperate climate but with a fauna and flora different from that of England or Western Europe. Such countries are obviously those from which we are likely to receive some new and dangerous pest, and the second inspection gives an opportunity of detecting unusual pests when they have developed sufficiently to become visible but before any serious spread has taken place. The last measure which is needed to reinforce the inspection—and indeed any other—system for dealing with plant imports is the organisation of a sufficient plant pathological service, so that diseases and pests which have crept in in spite of all precautions may be detected before it is too late to adopt drastic measures against them. So far as England and Wales is concerned this service is rendered first by the Ministry's

inspectors who are constantly out in the field, and secondly by the entomologists and mycologists, both advisers and others, who are attached to colleges and research stations and who are in touch both with farmers and fruit growers and with county educational staffs. In this way over 60 trained observers are available for the detection of new pests and diseases, but whether they are sufficient or not, time alone will tell. The most recent case of attempted colonisation by a foreign pest is the outbreak of the Chrysanthemum Midge[1], detected by the Lea Valley Experiment Station, and it is satisfactory to note that the outbreak appears to be of such proportions that the definite eradication of the species may reasonably be expected. It would not be right, however, to end on too optimistic a note, for it cannot be too widely realised that there is probably no system of regulating plant imports, even if it be carried to the extent of preventing trade in plants, which will eliminate the gradual addition of new pests and diseases to the list of those already known in the country. Such additions can be restricted by the adoption of a sound system of regulating plant imports, they may be even more restricted if all concerned combine to render any system, regardless of the nature, effective, but some warning is perhaps desirable that in spite of all that can be done new pests will continue to creep in probably until in the long lapse of time every pest or disease has extended throughout its potential geographical range. For the final means of defence, therefore, it is necessary to look not to systems of administration, but rather to a steadily increasing knowledge of the methods of fighting plant pests and diseases, a point at which the present subject may safely be concluded.

[1] *Diarthronomyia hypogaea* F. Löw.

REPORT OF THE COUNCIL FOR THE YEAR 1927

During 1927 the Association has met on eight occasions. At five of these various subjects of interest were brought before the Association by members and visitors to whom the Association is greatly indebted. The subjects included Insecticides, Plant Alkaloids, Tropical African Agriculture, Foot and Mouth Disease, and Insectivorous Plants. This last was in substitution for a Forestry programme which had to be abandoned owing to the illness of one of the members taking part. At the Annual General Meeting a series of demonstrations and exhibits was arranged; in March the Association had the privilege of visiting the Imperial Institute; and in June a two-day provincial meeting was held at the South-Eastern Agricultural College, Wye, by the courteous invitation of the Principal.

The attendance at meetings has varied from 40 to 87, the average being 58. The proportion of Members to Visitors has been about 75 per cent.

During the year the Association has lost 5 Members through resignation, and the Council have, with regret, to record the death of Mr H. G. Billinghurst. The Council, with great regret, have also to record the death of an Honorary Member, namely, Prof. Antonio Berlese, who died on the 24th October last after a short illness following an accident. Prof. Berlese was Director of the Research Station for Agricultural Entomology at Florence, and an entomologist of world-wide reputation. Against this total decrease in membership of 7 the Association has had the pleasure of electing 25 new Members. The net result is an increase of 18 in membership and the Association now numbers 255 Honorary and Ordinary Members.

The Royal Microscopical Society kindly invited the Association to be represented at its meeting in Liverpool in March last. On the invitation of the Council, Mr J. C. Waller represented the Association.

The Council made a further annual contribution of £5 to the fund for maintaining the publication of the *Zoological Record*.

At the last Annual General Meeting the Association approved the Council's recommendation that the Association should be registered as a Company without the word "limited." When action came to be taken on this decision it was found that the expense entailed would be approximately £100. The Council thereupon made enquiries as to the cost of Incorporation by Royal Charter and ascertained that this would be considerably more expensive. After careful consideration the Council are of the opinion that any benefits likely to be obtained by the Association through registration or incorporation are not, at present, commensurate with the cost involved. They decided, therefore, to postpone action until the further wishes of the Association could be ascertained. The Council recommend to the Association, that no further action be taken in this matter at present.

On representation being made to the Council, it was decided that the card of the Association's meetings for the year should, in future, be sent to all Members, and that it should contain a notice asking Overseas Members to notify the Secretary when they would be in England and give their English addresses. The Secretary

would then notify Members of meetings falling within the period of their visits to this country.

During the past year the Association has again been so fortunate as to enjoy the hospitality of the Imperial College of Science and Technology for their meetings. The Council feel sure that the Association will like to take this opportunity of recording its grateful thanks for this most valued assistance.

Papers read to the Association during the year 1927:

Feb. 15*th.* Dr F. Tattersfield and Mr C. T. Gimingham: "Laboratory and Field Experiments on Contact Insecticides."

May 13*th.* Lt.-Colonel A. T. Gage: "The Principal Plants yielding Alkaloids." Dr T. A. Henry: "The Biochemistry of the Alkaloids." Dr J. Trevan: "The Medical Aspects of the Alkaloids."

June 18*th.* Prof. E. S. Salmon: "Economic Mycology at an Agricultural College 1906–1927." Dr W. Goodwin: "Sulphur-containing Sprays." Mr S. T. Parkinson: "Quantity Spacing and Depth of Sowing of Cereals." Rev. Dr Brade-Birks: "Economic Status of Millipedes and Centipedes." Mr V. C. Fishwick: "Research in Pig Husbandry."

Oct. 28*th.* Dr E. J. Butler: "Planting Developments and Difficulties in Nyasaland.' Mr W. Nowell: "The Work of the Amani Institute."

Nov. 18*th.* Dr F. C. Minett: "Foot and Mouth Disease in Farm Animals." Dr J. A. Arkwright: "Experimental Foot and Mouth Disease in Small Animals." Dr S. P. Bedson: "Physical Properties of the Virus, Filtration, etc. Foot and Mouth Disease." Mrs Y. M. Burbury: "Survival of the Virus outside the Body " Mr I. A. Galloway: "Lesions of Foot and Mouth Disease in Guinea-pigs."

Dec. 16*th.* Dr G. H. Rodman: "Insectivorous plants and how they live."

REPORT OF THE HON. TREASURER FOR THE YEAR 1927

A statement of the accounts of the Association for the year ending December 31st, 1927 appears on p. 332. During the year current subscriptions received amounted to £252. 9*s*. 8*d*. as compared with £252. 14*s*. 2*d*. for the previous year. Arrears amounting to £15. 7*s*. 0*d*. were received and subscriptions considered good, as yet unpaid, amounted to £37. 10*s*. 0*d*. It is necessary again to urge upon members to support the Association by prompt payment of contributions due from them. The working expenses of the Association have been considerably higher than for 1926. Cost of teas to members after the meetings amounted to £10. 5*s*. 9*d*. and the *Annals of Applied Biology*, vol. XIII, has entailed a sum of £408. 15*s*. 8*d*. for costs of publication after all receipts for the sales, etc. have been deducted. The amount required to meet the cost of vol. XIV is £175. 16*s*. 5*d*. or a reduction of £232. 19*s*. 3*d*., which is largely due to increased sales of back volumes and parts and of reprints of separate articles. The sum acquired for vol. XIV is paid out of 1928 revenues, but as the cash balance at the bank at the end of the current year covers this requirement more than twice over, the financial position of the Association may be regarded as being satisfactory.

A. D. IMMS,
Hon. Treasurer.

TREASURER'S STATEMENT FOR THE YEAR ENDING DECEMBER 31ST, 1927

CASH ACCOUNT.

Cr	£	*s.*	*d.*	*Dr*	£	*s.*	*d.*
Jan. 1. Cash at Bank . . .	71	13	3	Postage	7	0	7
Dec. 31. Subscriptions:				Stationery and minor printing	3	15	7
A. Current . . .	252	9	8	Treasurer	415	1	0
B. Arrears . . .	15	7	0	Secretaries	26	10	6
C. Advances . .	10	0	0	Balance at Bank	126	18	2
Entrance Fees . .	13	2	6	Placed on Deposit	200	0	0
Contributions to cost of papers in *Annals*	12	0	0				
Bank Interest . . .	4	13	5				
Taken from Deposit .	400	0	0				
Total	£779	5	10	Total	£779	5	10

BALANCE SHEET.

Liabilities.	£	*s.*	*d.*	Assets.	£	*s.*	*d.*
Subscriptions in advance . .	10	0	0	Current a/c	126	18	2
Liability on *Annals*, vol. XIV .	175	16	5	Deposit a/c	230	0	0
Excess of assets over liabilities	770	0	3	Subscriptions two years or less in arrears and considered good	37	10	0
				National Savings' Certificates .	506	5	0
				Estimated value of stock of *Annals of Applied Biology* with publishers	55	3	6
Total	£955	16	8	Total	£955	16	8

A. D. IMMS, *Hon. Treasurer.*

We have examined the Treasurer's statement of expenditure and receipts and have found it correct. We consider that the above balance sheet correctly represents the position of the Association.

C. T. GIMINGHAM.
GEO. H. PETHYBRIDGE.

Jan. 16th, 1928.